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Remarks

The above Amendments and these Remarks are in reply to the Office Actions mailed April 16, 2003 and August 1, 2003.

Applicants have included claims 1-10, which had been previously cancelled.

Claims 32 and 36 have been amended to correct typographical errors, and thus are not narrowing amendments. Claims 38-44 have been amended to correct antecedents and are be not narrowing amendments.

Claims 11-16, 24-27, 30-31 and 36-46 stand rejected under 35 U.S.C. §102(b) as anticipated and/or obvious under 35 U.S.C. §103 by Sara et al. (EP 0366638; published 02.05.90 "Sara 1") alone or in combination with the instant specification at pages 1-2 to demonstrate inherency e.g., damage/loss of glial cells resulting from [due to] neural damage/injury e.g., from asphyxia/ischemia/hypoxia/stroke, and dementia disorders such as Alzheimer's addressing non-dopaminergic neurons. Office Action, page 4, paragraph 7. Additionally, "Thus, the reference treatment of neurodegenerative/neurocatabolic disease states and ischemic brain damage (e.g., stroke and asphyxia) addresses the treatment of injuries or disease which result in neural cell death." Office Action, page 7 bridging to page 8. Applicants respectfully traverse the rejections.

I. Anticipation

Applicants submit that Sara 1 does not anticipate, either expressly or inherently, the instant claims. MPEP 2131 states:

> A claim is anticipated only if **each and every element as set forth in the claim** is found, either expressly or inherently described, in a single prior art reference [references omitted]. The **identical invention must be shown in as complete detail** as is contained in the ... claim [reference omitted]. Emphasis added.

Express Anticipation

Sara 1 does not disclose "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease comprising the step of administering to said mammal a neuroprotective amount of . . . GPE, " and therefore doesn't expressly disclose "each and every element as set forth in the claim" as required. The Examiner's statement "[t]hus, the reference

treatment of neurodegenerative/neurocatabolic disease states and ischemic brain damage (e.g., stroke and asphyxia) addresses the treatment of injuries or disease which result in neural cell death" is unclear. Applicant does not understand whether the term "addresses" was intended to mean "anticipates." Clarification is requested. However, regardless of the meaning of the term "addresses," Applicants assert that altering neurotransmitter release induced by cellular depolarization does not necessarily include treating "injuries or disease which results in neural cell death."Thus, Applicants respectfully submit that Sara 1 does not expressly disclose each and every element of claim 11.

Inherent Anticipation

Applicants submit that Sara 1 does not inherently anticipate the instant claims. MPEP 2112 states:

> To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is **necessarily present** in the thing described in the reference, and that **it would be so recognized** by persons of ordinary skill. **Inherency, however, may not be established by probabilities or possibilities.** The mere fact that a certain thing may result from a given set of circumstances is not sufficient. Emphasis added.

Firstly, Applicant's claims are drawn to "methods for treating" and not to "compositions." Applicants note that a claim is drawn to an "invention" and that invention requires a conception and reduction to practice. Applicants note that the discovery of a new effect or new use of a known composition results in a new "conception" and thus a new "invention" that is not necessarily unpatentable. Thus, Applicants' discovery of "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease comprising the step of administering to said mammal a neuroprotective amount of ... GPE" is not necessarily rendered unpatentable by prior art disclosing either GPE or other uses of GPE.

Missing Elements Defeat Anticipation by Inherency

Applicants respectfully submit that there are elements missing from the prior art necessary to link a "neuromodulator" effect of Sara 1 and "neuroprotective" effects of the instant application.

Specifically, the words "neuromodulator" and "neuroprotective" do not have the same plain meanings, and are used differently in the documents themselves. Applicants enclose as Appendix I, copies of relevant pages of the Random House Unabridged Dictionary (Second Edition).

Plain Meanings of "Neuromodulator" and "Neuroprotective"

The term "neuromodulator" is a compound word made of the prefix "neuro," which is understood by persons in the art to refer to neurons. The remainder of the word is "modulator" which is subject to definition by referring to its plain meaning as defined in dictionaries.

The Random House Unabridged Dictionary (Second Edition) defines "modulator" to mean: "A person or thing that **modulates.**"

The word "modulate" means:

> (1) **to regulate by or adjust to a certain measure or proportion**; soften; tone down. (2) to alter or adapt (the voice) according to the circumstances. (3) *Music* (a) to attune to a certain pitch or key. (b) to vary the volume of (tone). (4) *Telecommunications*: (a) to cause the amplitude, frequency, phase, or intensity of (a carrier wave) to vary in accordance with a sound wave or other signal, the frequency of the signal wave usually being very much lower than that of the carrier. . . Emphasis added.

Likewise, the words "neuroprotective" and "neuroprotection" are compound words consisting of the prefix "neuro" and the remainder being either "protective" or "protection." The Random House Unabridged Dictionary (Second Edition) defines "protection" to mean:

> (1) the act of protecting or the state of being protected; **preservation from injury or harm.** (2) **a thing, person, or group that protects**: *This vaccine is a protection against disease. . . .*" Emphasis added.

Applicants note that the dictionary does not list either as a synonym of the other. Thus, the two terms "modulate" and "protect" have different plain meanings.

Applicants submit that the word "neuromodulate" as used in Sara 1, most closely fits with the definition above "to regulate by or adjust to a certain measure or proportion." The Experiments described in Sara 1 demonstrate that GPE can "regulate or adjust" the function of neurons in brain slices by increasing or inhibiting the release of acetylcholine or by increasing the spinal reflex response.

Applicants also submit that the meaning of the claim limitation "neuroprotective amount of... GPE" means an amount of GPE that is "neuroprotective." Applicants further submit that the definition described above applies to the claim language: "preservation from injury or harm" or "a thing.. that protects."

The Terms "Neuromodulator" and "Neuroprotective" are Used Differently By Sara and by Applicants

Sara 1 uses the term "neuromodulator" in relation to results of **acute**, *in vitro* studies on brain slices (e.g., Example 2), in which GPE "is a **modulator** of neural function, thereby **stimulating or inhibiting** neural activity." Col. 1, lines 36-37; emphasis added. Sara 1 also discloses results of studies showing potentiation of spinal cord reflexes by GPE. Applicants assert that this use of "neuromodulator" is very close to the plain meaning above, namely "to regulate [neurotransmitter release or spinal reflexes] by or adjust to a certain measure or proportion [e.g., by GPE]."

Additionally, another publication by Sara, after the publication of Sara 1 sheds light on the meaning of the term "neuromodulator." In the article, "Neuroactive Products of IGF-1 and IGF-2 Gene Expression in the CNS" Molecular Biology and Physiology of Insulin and Insulin-Like Growth Factors, Plenum Press, New York; pp 439 - 448 (1991)("Sara 3; copy provided herewith as Appendix III) provides insight into the meaning of the term as used in Sara 1.

> GPE is believed to act as a **neuromodulator regulating neurotransmission**. GPE is the first example of the product of a growth factor gene having a **specific role in neurotransmission**. Page 443; emphasis added.

Applicants can find no indication in Sara 3 that the term "neuromodulation" had any other meaning, including meaning "neuroprotective" or any other term relating to enhancing survival of "glial cells or non-dopaminergic neurons." Applicants therefore respectfully submit that the term "neuromodulator" means a "neuromodulator regulating neurotransmission." Applicants submit that the term "neurotransmission" is understood by those of skill in the art to refer to the release of neurotransmitters from neurons and the actions of those neurotransmitters on post-synaptic neurons and does not refer to an ability to promote growth or survival of neurons or other cells.

In contrast, Applicants use the term "neuroprotective" refers to inhibition of cell death, as pointed out in claim 11: "A method for **pr tecting** glial **cells** or non-dopaminergic neural cells in a

mammal **against death** from neural injury or disease comprising the step of administering to said mammal a **neur protective** amount of... GPE..." Emphasis added. Applicants submit that their use of "neuroprotection" is close to the above dictionary definition: "the act of protecting or the state of being protected; **preservation from injury or harm**. (2) **a thing, person, or group that protects**: *This vaccine is a protection against disease. . . .*" [Emphasis added, italics in original.]

Additionally, for Sara 1 to inherently anticipate the instant claims, persons of ordinary skill would have to believe that **both** increasing **and** decreasing neurotransmitter release are **necessarily** linked to "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease." If the Examiner is aware of any evidence of such a reasonable belief, he is requested to provide such evidence, through either a prior art reference if available, or an Affidavit or a Declaration.

Further, Applicants invite consideration of what is not disclosed in Sara 1. Although Sara 1 discusses **potential** uses of GPE to treat dementias, but Sara 1 does not provide an enabling disclosure of any such use. Sara 1 discloses no experiments on neural survival. Sara 1 discloses (1) no *in vivo* experiments in which GPE was used, (2) no long-term studies of any effect of GPE, (3) no experiments in which neural survival *in vivo* or *in vitro* was measured, and (4) no link between **acute** *in vitro* studies on acetylcholine release or spinal cord reflexes and survival of any cell type. Further, (5) none of the studies were described as being on brain slices from any animal that had been subjected to neural damage or disease. Thus, Applicants conclude that Sara 1 did not describe any conception and reduction to practice of any "neuroprotective" effect of GPE, and therefore cannot anticipate the instant claims.

Finally, the Examiner has provided no evidence that necessarily links "stimulating or inhibiting neural activity" with "protecting glial cells or non-dopaminergic neural cells in a mammal against **death** from neural injury or disease" as in claim 11. [Emphasis added.]

Therefore, Applicants submit that Sara 1 cannot inherently indicate that neuromodulation is useful for "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease" Although it may be possible that such an effect exists, such a **possibility** cannot sustain a rejection based on inherency. "Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of

circumstances is not sufficient." MPEP, Id. Thus, Applicants submit that a *prima facie* case for anticipation has not been made.

In light of the dearth of enabling disclosure about roles of GPE on neuroprotection, Applicants submit that Sara 1 cannot anticipate the instant claims.

II. Obviousness

A. Sara

Claims 11-17, 24-27, 30-31 and 36-46 stand rejected under 35 U.S.C. §103 as obvious over Sara (Sara 1).

> To establish a *prima facie* case for obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a **reasonable expectation of success**. Finally, the prior art reference must **teach or suggest all the claim limitations**. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and **not based on applicant's disclosure**.

Applicants respectfully submit that the instant claims cannot be rendered obvious by Sara 1. As described above, Sara 1 discloses that GPE either "stimulates or inhibits" neural activity and can potentiate spinal cord reflexes. However, Sara 1 neither teaches nor suggests that GPE can be effective in "protecting glial cells or non-dopaminergic neural cells in a mammal against **death** from neural injury or disease." [Emphasis added.] Applicants therefore submit that Sara 1 cannot render the instant claims obvious.

Thus, at best, the experiments disclosed in Sara 1 provide an "invitation to experiment" on possible effects of GPE on brain slices from brain-damaged or brain-diseased animals, but could not have provided a reasonable basis to conclude that acute effects of GPE on neurotransmitter release inherently discloses any property of GPE to promote "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease." Rather, for Sara 1 to render the instant claims obvious, **both** potentiation and inhibition of acetylcholine release would have to relate to "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease". No link between neurotransmitter release and either disease or cell death

was made in Sara 1, nor was there any disclose of conception or understanding that intervention using GPE could result in decreased neural cell death (neuroprotection).

Regarding the fact that a *prima facie* case for obviousness requires a "motive to modify the reference" and "reasonable likelihood of success," Applicants note that a subsequently published article by Sara ("The Biological Role of Truncated Insulin-like Growth Factor-1 and the Tripeptide GPE in the Central Nervous System" Annals of the New York Academy of Science; pp: 183 - 191 (1991); "Sara 2"; copy enclosed in Appendix II) addresses similar issues as in the Sara EP 0366638 ("Sara 1") but actually teaches away from Applicants' claims. In particular, Sara 2 states:

> Extensive *in vivo* studies have **not revealed any growth-promoting activity of GPE**. . . . As shown in Figure 4, no significant growth effects, including tail length and organ weights, were observed. Page 187, middle of first full paragraph.

Thus, Applicants submit that at the time of publication of Sara 2, the first inventor of Sara 1 (Sara) could not have had a reasonable belief that GPE could be a growth modulator, and thus that there would be neither a motive to try nor a reasonable likelihood of success at achieving the Applicants' invention. In the absence of a reasonable belief by the primary inventor, Applicants submit that no person of ordinary skill could have such a reasonable belief. Applicants submit that both Sara 1 and Sara 2 considered GPE to be an agent that acted on neurotransmitter receptors and not as a growth promoting hormone. Because Sara 1 was silent about any effects of GPE to promote "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease," Applicants submit that at the time of publication of Sara 1, there was neither motive nor a reasonable belief that GPE could so act.

Additionally, Sara 3 provides insight into the teaching of Sara 1. In particular:

> The peptide products from expression of the IGF-1 gene in the brain, namely truncated IGF-1 and GPE, appear to induce biological responses via two separate mechanisms. The action of truncated IGF-1 is mediated via the IGF-1 receptor. GPE does not cross-react with the IGF-1 receptor, but rather in the NMDA receptor, and possibly an additional, as yet undefined, mechanism.
>
> ...
>
> Instead, GPE cross-reacts in the NMDA (N-methyl-D-aspartate) receptor which is a subtype of receptors for the major excitatory amino acid neurotransmitter glutamate.. . . .GPE potentiates the release of dopamine via interaction in the NMDA receptor.

...

> In conclusion, . . . GPE is believed to act as a **neuromodulator regulating neurotransmission**. GPE is the first example of the product of a growth factor gene having a **specific role in neurotransmission.** Page 443; emphasis added.

Thus, as with Sara 1 and Sara 2, Sara 3 neither teaches nor suggests any role of GPE for "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease" or for providing any other growth-promoting effect on any cell type, including neurons or glial cells.

Rather, Applicants respectfully submit that the motive and reasonable likelihood of success were provided by the Applicant's own instant disclosure.

B. Sara in View of Sibalis

Claims 11-17, 24-27 , 30-31 and 36-46 stand rejected under 35 U.S.C.§103 over Sara (Sara 1) in view of Sibalis (U.S. 5,032,109; "Sibalis").

Applicants incorporate herein the discussions presented above for Sara 1 .

The Examiner stated that Sibalis teaches transdermal delivery of "polypeptides containing about three to 20 alphaamino acid units." However, Applicants can find no teaching in Sibalis and Sara 1 together of any method for "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease." Thus, the combination of Sara 1 and Sibalis does not disclose all the limitations of the pending claims with a reasonable likelihood of success, and thus cannot render Applicants' claims obvious. Applicants therefore urge the Examiner to reconsider the rejection and find the claims allowable.

C. Sara in View of Gluckman

Claims 11-16 and 18-46 stand rejected under 35 U.S.C. §103 over Sara (Sara 1) in view of Gluckman (WO 93/02695; "Gluckman").

Applicants incorporate herein the discussions presented above for Sara 1.

The Examiner stated that Gluckman teaches "a method for treatment or prevention of CNS damage caused by neurodegenerative disease and trauma which primarily causes damage to glia and/or other non-cholinergic cells in the CNS." Office Action, page 9, bottom paragraph. The Examiner also stated "It is noteworthy that the Gly-Pro-Glu peptide, as presently claimed, is derived

from the N-terminal three amino acids of IGF-1 peptide." Office Action, page 10 bottom of first paragraph.

Applicants note that Gluckman does not disclose "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease, comprising administering a neuroprotective amount of ... GPE ..." as in claim 11. Nowhere in either Sara 1 nor Gluckman, nor in the combination of Sara 1 and Gluckman together, is any teaching of the use of GPE as in claim 11. Thus, the combination of Sara 1 and Gluckman does not disclose all the limitations of the pending claims with a reasonable likelihood of success, and thus cannot render Applicants' claims obvious.

Although GPE is the N-terminal tripeptide of IGF-1, both Sara 2 and Gluckman teach away from GPE as a neuroprotective agent. First, Gluckman teaches that IGF-1 is neuroprotective (e.g., see Abstract and Summary of the Invention, page 3, first paragraph). Next, Sara 2 states: "The aminoterminal tripeptide of IGF-1, GPE, displays a different range of biological actions compared to truncated IGF-1. These effects are not mediated by IGF-1 receptors. As shown in Figure 3, GPE fails to cross-react with the IGF-1 receptor and does not influence the binding of either intact or truncated IGF1 to the receptor." Page 187, middle paragraph, middle section. Thus, Applicants submit that one or ordinary skill in the art would view Sara 1 in the same light as Sara 2, and when combined with Gluckman, would provide no motive to nor a reasonable believe in the success of, any study to determine whether GPE had neuroprotective properties.

Rather, Applicants submit that the instant disclosure provided the link between IGF-1, GPE and "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease, comprising administering a neuroprotective amount of ... GPE ..." as in claim 11. "To date, there has been no enabling reference in the prior art to the manipulation of the cleaved tripeptide GPE itself to prevent or treat CNS injury or disease leading to CNS damage *in vivo*." Page 3, third paragraph. Using such hindsight reconstruction to argue for unpatentability is impermissible under 35 U.S.C. §103, the MPEP and case law. Applicants therefore urge the Examiner to reconsider the rejections and find the claims allowable.

III. Conclusions

Applicants respectfully submit that there is insufficient showing that Sara 1 either expressly or inherently anticipates or renders the instant claims obvious and that no *prima facie* for either

rejection has been made. Applicants respectfully request the Examiner to provide the missing evidence necessary to make a *prima facie* showing of either anticipation or obviousness. In the absence of such evidence, either through citation of a publication or through an Affidavit or Declaration, Applicants request the Examiner to reconsider the rejections and find the claims allowable.

Further, Applicants conclude that no combination of Sara 1, Sibalis or Gluckman taught or suggested, with a reasonable likelihood of success, all limitations of the instant claims, and therefore, that no combination of those references renders the instant claim obvious. In fact, Sara 2 actually taught away from the instant claims. Because Sara 2 was published after Sara 1, Applicants conclude that any interpretation of Sara 1 to teach "protecting glial cells or non-dopaminergic neural cells in a mammal against death from neural injury or disease, comprising administering a neuroprotective amount of ... GPE ..." is not supported.

In light of the above, it is respectfully submitted that all of the claims now pending in the subject patent application should be allowable, and a Notice of Allowance is requested. The Examiner is respectfully requested to telephone the undersigned if he [she] can assist in any way in expediting issuance of a patent.

The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit Account No. 06-1325 for any matter in connection with this response, including any fee for extension of time, which may be required.

Respectfully submitted,

Date: August 5, 2003

By: D. Benjamin Borson

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APPENDIX I

Copies of Relevant Pages from

Random House Unabridged Dictionary (Second Edition)

RANDOM HOUSE UNABRIDGED DICTIONARY

Second Edition

...tic variation.

...o-der-sohn-Beck·er (mō′dər zōn bek′ər), *n.* **Pau-** (pou′lä), 1876–1907, German painter

...od·est (mod′ist), *adj.* **1.** having or showing a mod...ate or humble estimate of one's merits, importance, ...c.; free from vanity, egotism, boastfulness, or great ...etensions. **2.** free from ostentation or showy extrava...nce: *a modest house.* **3.** having or showing regard for ...e decencies of behavior, speech, dress, etc.; decent: *a ...odest neckline on a dress.* **4.** limited or moderate in ...mount, extent, etc.: *a modest increase in salary.* [1555–...5; < L *modestus* restrained, decorous, equiv. to *modes-* ... of **modus*, an s-stem akin to *modus* MODE¹, perh. < ...*nedos*, with the vowel of *modus*; cf. *moderārī* to MOD...ERATE, from the same n. stem) + *-tus* adj. suffix] **...mod′est·ly**, *adv.*

—**Syn.** **1.** retiring, unassuming. **1, 2.** unpretentious, ...nobtrusive. **3.** pure, virtuous. MODEST, DEMURE, PRUD...ISH imply conformity to propriety and decorum, and a ...istaste for anything coarse or loud. MODEST implies a ...ecoming shyness, sobriety, and proper behavior: *a mod...st, self-respecting person.* DEMURE implies a bashful, ...uiet simplicity, staidness, and decorum; but can also in...icate an assumed or affected modesty: *a demure young ...horus girl.* PRUDISH suggests an exaggeratedly self-con...scious modesty or propriety in behavior or conversation ...f one who wishes to be thought of as easily shocked and ...ho often is intolerant: *a prudish objection to a harmless ...mark.* —**Ant.** **3.** bold, coarse.

...o·des·to (mə des′tō), *n.* a city in central California. ...06,105.

...od·es·ty (mod′ə stē), *n., pl.* **-ties.** **1.** the quality of ...eing modest; freedom from vanity, boastfulness, etc. **2.** ...egard for decency of behavior, speech, dress, etc. **3.** ...implicity; moderation. [1525–35; < L *modestia.* See ...ODEST, -Y³]

...od′esty pan′el, a panel across the front of a desk, ...sp. an office desk, designed to conceal the legs of a per...on seated at it.

...ODFET (mod′fet′), *n. Electronics.* modulation-doped ...ield effect transistor.

...odGk, Modern Greek. Also, **Mod. Gk., Mod. Gr.**

...odHeb, Modern Hebrew. Also, **Mod. Heb.**

...od·i·cum (mod′i kəm), *n.* a moderate or small ...mount: *He hasn't even a modicum of common sense.* ...1425–75; late ME < L, n. use of neut. of *modicus* moder...te, equiv. to *modi-*, comb. form of *modus* limit (see ...ODE¹) + *-cus* adj. suffix]

...odif., modification.

...od·i·fi·cand (mod′ə fi kand′), *n. Gram.* a word that ...s modified, or qualified, by another. In *red books, books* ...s a modificand. [1825–35; < L *modificandum* (a thing) ...o be measured or limited, ger. of *modificāre* to MODIFY]

...od·i·fi·ca·tion (mod′ə fi kā′shən), *n.* **1.** an act or ...nstance of modifying. **2.** the state of being modified; ...artial alteration. **3.** a modified form; variety. **4.** *Biol.* ...a change in a living organism acquired from its own ac...ivity or environment and not transmitted to its descend...nts. **5.** limitation or qualification. **6.** *Gram.* **a.** the use ...f a modifier in a construction, or of modifiers in a class ...f constructions or in a language. **b.** the meaning of a ...nodifier, esp. as it affects the meaning of the word or ...ther form modified: *Limitation is one kind of modifica...ion.* **c.** a change in the phonological shape of a mor...pheme, word, or other form when it functions as an ele...ment in a construction, as the change of *not* to *-n't* in ...*doesn't.* **d.** an adjustment in the form of a word as it ...passes from one language to another. [1495–1505; < L ...*modificātiōn-* (s. of *modificātiō*), equiv. to *modificāt(us)* ...ptp. of *modificāre*; see MODIFY) + *-iōn-* -ION]

...nod·i·fi·ca·to·ry (mod′ə fi kə tôr′ē, -tōr′ē), *adj.* ...modifying. Also, **mod′i·fi·ca′tive.** [1815–25; < L ...*modificāt(us)* (see MODIFICATION) + -ORY¹]

...nod′ified Amer′ican plan′, (in hotels) a system of ...paying a single fixed rate that covers room, breakfast, ...and one other meal, usually dinner. *Abbr.:* MAP Cf. **American plan, demi-pension, European plan.**

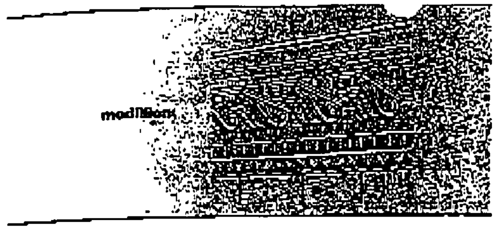
...nod·i·fi·er (mod′ə fī′ər), *n.* **1.** a person or thing that ...modifies. **2.** *Gram.* **a.** a word, phrase, or sentence ele...ment that limits or qualifies the sense of another word, phrase, or element in the same construction. **b.** the im...mediate constituent of an endocentric construction that is not the head. [1575–85; MODIFY + -ER¹]

—**Usage.** See **dangling participle, misplaced modifier.**

nod·i·fy (mod′ə fī′), *v.,* **-fied, -fy·ing.** —*v.t.* **1.** to change somewhat the form or qualities of; alter partially; amend: *to modify a contract.* **2.** *Gram.* (of a word, phrase, or clause) to stand in a syntactically subordinate relation to (another word, phrase, or clause), usually with descriptive, limiting, or particularizing meaning; be a modifier. In *a good man, good* modifies *man.* **3.** to be the modifier or attribute of. **4.** to change (a vowel) by umlaut. **5.** to reduce or lessen in degree or extent; mod...erate; soften: *to modify one's demands.* —*v.i.* **6.** to be or become modified. [1350–1400; ME *modifien* < MF *modifier* < L *modificāre* to impose a rule or pattern, regulate, restrain. See MODE¹, -IFY] —**mod′i·fi′a·ble**, *adj.* —**mod′i·fi′a·bil′i·ty, mod′i·fi′a·ble·ness,** *n.*

—**Syn.** **1.** vary, adjust, shape, reform. **5.** MODIFY, QUALIFY, TEMPER suggest altering an original statement, condition, or the like, so as to avoid anything excessive or extreme. To MODIFY is to alter in one or more particu...lars, generally in the direction of leniency or moderation: *to modify demands, rates.* To QUALIFY is to restrict or limit by exceptions or conditions: *to qualify one's praise.*

VL **multitionem*, var. of ... See MUTULE, -ION]


modillion

mo·di·o·lus (mō dī′ə ləs, mə-), *n., pl.* **-li** (-lī′). *Anat.* the central, conical axis of the cochlea of the ear. [1685–95; < NL, L: nave of a wheel bucket, drinking vessel, equiv. to *modi(us)* a dry measure (perh. deriv. of *modus* MODE¹) + *-olus* -OLE¹] —**mo·di′o·lar**, *adj.*

mod·ish (mō′dish), *adj.* in the current fashion; stylish. [1650–60; MODE² + -ISH¹] —**mod′ish·ly**, *adv.* —**mod′·ish·ness**, *n.*

—**Syn.** smart, chic, fashionable, trendy.

mo·diste (mō dēst′; *Fr.* mô dēst′), *n., pl.* **-distes** (-dēsts′; *Fr.* -dēst′). *Older Use.* a female maker of or dealer in women's fashionable attire. [1830–40; < F; see MODE², -IST]

Mo·djes·ka (mō jes′kə), *n.* **He·le·na** (hə lā′nə), (*Helena Opid Modrzejewska*), 1840–1909, Polish actress, in U.S. after 1876.

Mo·doc (mō′dok), *n., pl.* **-docs,** (*esp. collectively*) **-doc.** a member of an American Indian people belonging to the Lutuamian group and ranging from southern Oregon to northern California.

mo′dock wool′ (mō′dok). See **territory wool.** [special use of MODOC]

mod. praesc., (in prescriptions) in the manner prescribed; as directed. [< L *modō praescriptō*]

Mo·dred (mō′drid), *n. Arthurian Romance.* the nephew and treacherous killer of Arthur. Also, **Mordred.**

mod·u·lar (moj′ə lər), *adj.* **1.** of or pertaining to a module or a modulus. **2.** composed of standardized units or sections for easy construction or flexible arrangement: *a modular home; a modular sofa.* **3.** *Math.* (of a lattice) having the property that for any two elements with one less than the other, the union of the smaller element with the intersection of the larger element and any third element of the lattice is equal to the intersection of the larger element with the union of the smaller element and the third element. **4.** *Computers.* composed of software or hardware modules that can be altered or replaced without affecting the remainder of the system. —*n.* **5.** something, as a house or piece of furniture, built or organized in self-contained units or sections. **6.** a self-contained unit or item, as of furniture, that can be combined or interchanged with others like it to create different shapes or designs. [1790–1800; < NL *modulāris.* See MODULE, -AR¹]

mod′ular arith′metic, arithmetic in which numbers that are congruent modulo a given number are treated as the same. Cf. **congruence** (def. 2), **modulo, modulus** (def. 2b). [1955–60]

mod·u·lar·i·ty (moj′ə lar′i tē, mod′yə-), *n.* the use of individually distinct functional units, as in assembling an electronic or mechanical system. [1935–40; MODULAR + -ITY]

mod·u·lar·ize (moj′ə lə rīz′), *v.t.,* **-ized, -iz·ing.** to form or organize into modules, as for flexibility. Also, *esp. Brit.,* **mod′u·lar·ise′.** [1955–60; MODULAR + -IZE] —**mod′u·lar·i·za′tion**, *n.*

mod·u·late (moj′ə lāt′), *v.,* **-lat·ed, -lat·ing.** —*v.t.* **1.** to regulate by or adjust to a certain measure or proportion; soften; tone down. **2.** to alter or adapt (the voice) according to the circumstances, one's listener, etc. **3.** *Music.* **a.** to attune to a certain pitch or key. **b.** to vary the volume of (tone). **4.** *Telecommunications.* to cause the amplitude, frequency, phase, or intensity of (a carrier wave) to vary in accordance with a sound wave or other signal, the frequency of the signal wave usually being very much lower than that of the carrier. —*v.i.* **5.** *Telecommunications.* **a.** to modulate a carrier wave. **b.** *CB Slang.* to talk; visit: *Enjoyed modulating with you.* **6.** *Music.* to pass from one key to another: *to modulate abruptly from A to B flat.* [1650–60; < L *modulātus* (ptp. of *modulārī* to regulate (sounds), set to music, play an instrument). See MODULE, -ATE¹] —**mod·u·la·bil·i·ty** (moj′ə lə bil′i tē), *n.* —**mod′u·la′tive, mod·u·la·to·ry** (moj′ə lə tôr′ē, -tōr′ē), *adj.*

—**Syn.** **2.** temper, control.

mod·u·la·tion (moj′ə lā′shən, mod′yə-), *n.* **1.** the act of modulating. **2.** the state of being modulated. **3.** *Music.* transition from one key to another. **4.** *Gram.* **a.** the use of a particular distribution of stress or pitch in a construction, as the use of rising pitch on *here* in *John is here?* **b.** the feature of a construction resulting from such use. [1350–1400; ME < L *modulātiōn-* (s. of *modulātiō*) rhythmical measure. See MODULATE, -ION]

mod·u·la·tor (moj′ə lā′tər), *n.* **1.** a person or thing that modulates. **2.** *Telecommunications.* a device for modulating a carrier wave. [1490–1500; < L *modulātor;* see MODULATE, -TOR]

mod·ule (moj′ōōl), *n.* **1.** a separable component, fre-

...any two operators and any group element ... having the first operator act on the element ... second element, and the second operator act ... ond element is equal to the result of having a ... erator, formed by adding or multiplying the ... tors, act on the first element. Cf. **ring¹** (d... *Computers.* **a.** part of a program that perform... function. **b.** an interchangeable, plug-in hard... [1555–65; < L *modulus;* see MODULUS]

mod·u·lo (moj′ə lō′), *adv. Math.* with re... modulus: *6 is congruent to 11, modulo 5.* [18... NL *modulō*, abl. of L *modulus* MODULUS]

mod·u·lus (moj′ə ləs), *n., pl.* **-li** (-lī′). **1.** *Ph...* efficient pertaining to a physical property. **2** ... that number by which the logarithms in one ... multiplied to yield the logarithms in another. ... tity by which two given quantities can be ... yield the same remainders. **c.** See **absol...** [1555–65; < L: a unit of measure; see MODE¹,

mod′ulus of elastic′ity, *Physics.* any of ... efficients of elasticity of a body, expressing th... tween a stress or force per unit area that acts ... the body and the corresponding fractional d... caused by the stress. Also called **coefficient ... ity, elastic modulus.** [1800–10]

mod′ulus of rigid′ity, *Physics.* See **shea...** [1875–80]

mod′ulus of tor′sion, *Physics.* See **shea...**

mo·dus op·e·ran·di (mō′dəs op′ə ran′dē ... mō′dōōs ô′pe rän′dē), *pl.* **mo·di op·e·ran...** op′ə ran′dē, mō′dī op′ə ran′dī; *Lat.* mō′dē ... dē). mode of operating or working. [164... *modus operandi*]

mo·dus vi·ven·di (mō′dəs vi ven′dē, -dī), **vi·ven·di** (mō′dē vi ven′dē, mō′dī vi ven′dī, ... ner of living; way of life; lifestyle. **2.** a tem... rangement between persons or parties pendin... ment of matters in debate. [1875–80 < L *moc...* mode of living]

Moe (mō), *n.* a male given name, form of Moses.

Moe·bi·us (mœ′bē əs, mā′-, mō′-), *n.* **Aug... nand.** See **Möbius, August Ferdinand.**

Moe·rae (mē′rē), *n.pl. Class. Myth.* the Fat...

Moe·si·a (mē′shē ə), *n.* an ancient country ... rope, S of the Danube and N of ancient Thrac... edonia: later a Roman province.

Moe·so·goth (mē′sō goth′, -sə-), *n.* one of ... tianized Goths who settled in Moesia in the 4... A.D.

Moe·so·goth·ic (mē′sō goth′ik, -sə-), *adj.* ... taining to the Moesogoths or their language ... GOTH + -IC]

mo·fette (mō fet′; *Fr.* mô fet′), *n.* **1.** a no... nation, consisting chiefly of carbon dioxide ... from the earth in regions of nearly extinct v... tivity. **2.** one of the openings or fissures from ... emanation issues. Also, **mof·fette′.** [1815–25 ... *moffetta* (Neapolitan *mufeta*), equiv. to *muff* ... It *mofa*) mould (< Langobardic; cf. G *Muff* ... MHG *müffeln* to give off a foul smell) + *-ett...*

mog¹ (mog), *v.,* **mogged, mog·ging.** *Dial.* ... move on, depart, or decamp (usually fol. by ... **2.** to walk or move along gently, slowly, an... —*v.t.* **3.** to cause to go from one place t... [1665–75; M(OVE) + (J)OG¹]

mog² (mog), *n.* moggy. [by shortening]

Mo·ga·di·shu (mō′gä dē′shōō), *n.* a seap... the capital of Somalia, in the S part. 400,000. ... **ga·di·scio** (mô′gä dē′shō).

Mog·a·dor (mog′ə dôr′, -dōr′; *Fr.* mô gA d... former name of **Essaouira.** **2.** (*l.c.*) Also, **mo...** a ribbed fabric of silk or rayon warp and cott... filling, used for neckties.

Mo·gen Da·vid (mō′gən dä′vid; *Seph. He...* dä vēd′; *Ashk. Heb.* mō′gən dō′vid), *Judc...* **Star of David.** [1900–05]

mog·gy (mog′ē), *n., pl.* **-gies.** *Brit. Inform...* Also, **mog.** [1815–25; said to be orig. Cockney ... derivations from dial. (W Midlands) *Moggy* pe... a calf, or from personal name MAGGIE, are d...

Mo·ghul (mō′gəl, -gul, mō gul′), *n., adj.* **M...** **1, 2, 6).**

Mo·gi das Cru·zes (mōō zhē′ däs krōō′z... in SE Brazil, E of São Paulo. 111,554.

mog·i·la·li·a (moj′ə lā′lē ə, -lāl′yə), *n.* any ... fect, as stuttering or stammering. Also, **mollal...** 80; < Gk *mogilál(os)* hardly talking (*mógi(s)* ... culty + *lálos* babbling) + *-ia* -IA]

Mo·gi·lev (mō′gi lef′; *Russ.* mə gyi lyôf′), *n* ... E Byelorussia (Belarus), on the Dnieper. 359,...

mo·go (mō′gō), *n., pl.* **-gos.** *Australian.* ... hatchet used by the Aborigines. [1815–25; ... *mu·gu*]

Mo·gol·lon (mō′gə yōn′), *n.* **1.** an extensi... or mesa in central Arizona; the southwestern ... the Colorado Plateau. **2.** a mountain range ... Mexico. —*adj.* **3.** *Archaeol.* of or pertaining to ... Indian culture of southeastern Arizona and sou... New Mexico 100 B.C.–A.D. 1000, characteriz... houses also used for burials and a distinctive ... white pottery decorated with human and anim...

; *prospectus carefully.* **2.** a brochure or other describing the major features, attractions, or a place, institution, or business to prospective ints, owners, or members. [1770–80; < L *prō-* ook, view, equiv. to *prōspec-*, s. of *prōspicere* + *-spicere*, comb. form of *specere* to look) + of *v.* action]

pros′par), *v.i.* **1.** to be successful or fortu- financial respects; thrive; flourish. —*v.t.* **2.** make successful or fortunate. [1425–75; late en < L *prosperāre* to make happy, deriv. of ROSPEROUS]

See **succeed.** —**Ant. 1.** fail.

ty (pro sper′i tē), *n., pl.* **-ties. 1.** a successing, or thriving condition, esp. in financial od fortune. **2. prosperities,** prosperous cir- [1175–1225; ME *prosperite* < OF < L *pro-* PROSPEROUS, -ITY]

(pros′pə rō′), *n.* (in Shakespeare's *The* exiled Duke of Milan, who is a magician.

us (pros′pər əs), *adj.* **1.** having or characnancial success or good fortune; flourishing; *a prosperous business.* **2.** well-to-do or *-osperous family.* **3.** favorable or propitious. ME < L *prosperus*] —**pros′per·ous·ly,** **′per·ous·ness,** *n.*

thriving. **2.** wealthy, rich. **3.** fortunate, lous.

) (*Gk.* prôs′fō rä; *Eng.* pros′fə rä′, -fər ə), h. antidoron. [1870–75; < Gk *prosphorá* an a bringing to, applying, equiv. to *pros-* to- *rá* something carried (verbid of *phérein* to

in (*Gk.* prôs′fō rôn; *Eng.* pros′fə ron′, *astern Ch.* an uncut loaf of altar bread besecrated. [< Gk *prósphoron,* n. use of neut. *s* useful, fitting, deriv. of *prosphorá* PROS-

), *v.i. Scot. and North Eng.* to exhibit pride ss; put on airs. [perh. Scots var., in v. use, —**pross′er,** *n.* —**pross′y,** *adj.*

), *n. Slang.* prostitute. [by shortening and

ros′ər), *n.* **Gabriel,** 1775?–1800, U.S. leader ul slave revolt.

), *interj.* prosit. [by contr.]

clin (pros′tə si′klin), *n. Biochem.* a pros- $_{20}$H$_{32}$O$_5$, that specifically inhibits the formaclots. [1975–80; PROSTA(TE) + CYCL(IC) + nodel of PROSTAGLANDIN]

·din (pros′tə glan′din), *n.* **1.** *Biochem.* s of unsaturated fatty acids that are in- contraction of smooth muscle, the control ion and body temperature, and many other functions. **2.** *Pharm.* any commercial of this substance. [1935–40; PROSTA(TE) + *d*²]

-s′tas), *n., pl.* **pro·sta·des** (prō stā′dēz). al architecture) an antechamber or vesti- a classical temple) the area included be- ides. [< Gk *prostás* lit., that which stands OSTASIS)]

(prō stā′sis), *n., pl.* **-ses** (-sēz). (in a classipronaos or prostas before a cella. [< Gk PRO-², STASIS]

ros′tāt), *Anat.* —*adj.* **1.** Also, **pros·tat·ic** of or pertaining to the prostate gland. —*n.* **ite gland.** [1640–50; < NL *prostata* < Gk standing before. See PRO-², -STAT]

to·my (pros′tə tek′tə mē), *n., pl.* **-mies.** n of part or all of the prostate gland. TATE + -ECTOMY]

and′, *Anat.* an organ that surrounds the les at the base of the bladder, comprising a ion, which controls the release of urine, ir portion, which secretes an alkaline fluid p part of the semen and enhances the ertility of sperm. [1890–40]

′tricle, *Anat.* a small pouch near the that opens into the urethra. [1920–25]

(pros′tə tiz′əm), *n.* symptoms of prossep. obstructed urination, arising from helent or chronic disease of the prostate 1900; PROSTATE + -ISM]

(pros′tə tī′tis), *n. Pathol.* inflammation gland. [PROSTATE + -ITIS]

1 (prō stûr′nəm), *n., pl.* **-na** (-nə), **-nums.** rite of the prothorax of an insect. [1820– PRO-¹, STERNUM] —**pro·ster′nal,** *adj.*

tion and fi[...] of prosthetic devices, esp. artificial limbs. [1890–95; PROSTHETIC, -ICS]

pros·the·tist (pros′thi tist), *n.* a person skilled in making or fitting prosthetic devices. [1900–05; PROSTHET(ICS) + -IST]

pros·thi·on (pros′thē on′), *n. Craniom.* the most forward projecting point of the anterior surface of the upper jaw, in the midsagittal plane. [1920–25; < Gk *prósthion,* neut. of *prósthios* frontal, akin to *prósthen* forward] —**pros′thi·on′ic,** *adj.*

pros·tho·don·tics (pros′thə don′tiks), *n.* (*used with a singular v.*) the branch of dentistry that deals with the restoration and maintenance of oral function by the replacement of missing teeth and other oral structures by artificial devices. Also, **pros·tho·don·tia** (pros′thə don′shə, -shē ə). [1945–50; PROSTH(ESIS) + -ODONT + -ICS]

pros·tho·don·tist (pros′thə don′tist), *n.* a specialist in prosthodontics. [1915–20; PROSTHODONT(ICS) + -IST]

pros·tie (pros′tē), *n. Slang.* a prostitute. [PROSTI(TUTE) + -IE]

Pro·stig·min (prō stig′min), *Pharm., Trademark.* a brand of neostigmine.

pros·ti·tute (pros′ti to͞ot′, -tyo͞ot′), *n., v.,* **-tut·ed, -tut·ing.** —*n.* **1.** a woman who engages in sexual intercourse for money; whore; harlot. **2.** a man who engages in sexual acts for money. **3.** a person who willingly uses his or her talent or ability in a base and unworthy way, usually for money. —*v.t.* **4.** to sell or offer (oneself) as a prostitute. **5.** to put to any base or unworthy use: *to prostitute one's talents.* [1520–30; < L *prōstitūta,* n. use of fem. of *prōstitūtus,* ptp. of *prōstituere* to expose (for sale), equiv. to *prō-* PRO-¹ + *-stitū-,* comb. form of var. s. of *statuere* to cause to stand + *-tus* ptp. suffix; see STATUS] —**pros′ti·tu′tor,** *n.*

—**Syn. 1.** call girl, streetwalker, courtesan; trollop, strumpet.

pros·ti·tu·tion (pros′ti to͞o′shən, -tyo͞o′-), *n.* **1.** the act or practice of engaging in sexual intercourse for money. **2.** base or unworthy use, as of talent or ability. [1545–55; < LL *prōstitūtiōn-* (s. of *prōstitūtiō*). See PROSTITUTE, -ION]

pro·sto·mi·ate (prō stō′mē āt′), *adj.* having a prostomium. [1885–90; PROSTOMI(UM) + -ATE¹]

pro·sto·mi·um (prō stō′mē əm), *n., pl.* **-mi·a** (-mē ə). the unsegmented, preoral portion of the head of certain lower invertebrates. [1866–70; < NL < Gk *prostómion* mouth. See PRO-², STOMA, -IUM] —**pro·sto′mi·al,** *adj.*

pro·sto·on (prō stō′on), *n., pl.* **-sto·a** (-stō′ə). (in classical architecture) a portico. [< Gk *próstoon;* see PRO-², STOA]

pros·trate (pros′trāt), *v.,* **-trat·ed, -trat·ing,** *adj.* —*v.t.* **1.** to cast (oneself) face down on the ground in humility, submission, or adoration. **2.** to lay flat, as on the ground. **3.** to throw down level with the ground. **4.** to overthrow, overcome, or reduce to helplessness. **5.** to reduce to physical weakness or exhaustion. —*adj.* **6.** lying flat or at full length, as on the ground. **7.** lying face down on the ground, as in token of humility, submission, or adoration. **8.** overthrown, overcome, or helpless: *a country left prostrate by natural disasters.* **9.** physically weak or exhausted. **10.** submissive. **11.** utterly dejected or depressed; disconsolate. **12.** *Bot.* (of a plant or stem) lying flat on the ground. [1350–1400; (adj.) ME *prostrat* < L *prōstrātus,* ptp. of *prōsternere* to throw prone, equiv. to *prō-* PRO-¹ + *strā-,* var. s. of *sternere* to stretch out + *-tus* ptp. suffix; (v.) ME *prostraten,* deriv. of the adj.] —**pros·tra·tive** (pros′trə tiv), *adj.* —**pros′tra·tor,** *n.*

—**Syn. 6.** prone, supine, recumbent.

pros·tra·tion (pro strā′shən), *n.* **1.** the act of prostrating. **2.** the state of being prostrated. **3.** extreme mental or emotional depression or dejection: *nervous prostration.* **4.** extreme physical weakness or exhaustion: *heat prostration.* [1520–30; < LL *prōstrātiōn-* (s. of *prōstrātiō*) a lying prone. See PROSTRATE, -ION]

pro·style (prō′stīl), *Archit.* —*adj.* **1.** (of a classical temple) having a portico on the front with the columns in front of the antae. —*n.* **2.** a prostyle building or portico. [1690–1700; (adj.) < L *prostylos* < Gk *próstylos* with pillars in front, equiv. to *pro-* PRO-² + *-stylos* -STYLE²; (n.) < Gk *próstylon,* n. use of neut. of *próstylos*]

pros·y (prō′zē), *adj.,* **pros·i·er, pros·i·est. 1.** of the nature of or resembling prose. **2.** prosaic; dull, tedious, wearisome, or commonplace. [1805–15; PROSE + -Y¹] —**pros′i·ly,** *adv.* —**pros′i·ness,** *n.*

pro·syl·lo·gism (prō sil′ə jiz′əm), *n. Logic.* a syllogism the conclusion of which is used as a premise of another syllogism; any of the syllogisms included in a polysyllogism except the last. Cf. **episyllogism.** [1575–85; < ML *prosyllogismus* < Gk *prosyllogismós.* See PRO-², SYLLOGISM]

See PROTO-, ANTAGONIST] —**pro·tag′o·nism,** *n.*

Pro·tag·o (prō tag′ər əs), *n.* c480–c421 B.C., Greek So[...] philosopher. —**Pro·tag·o·re·an** (prō tag′ə rē′ən), *adj.* —**Pro·tag′o·re′an·ism,** *n.*

prot·a·mine (prōt′ə mēn′, prō tam′in), *n. Biochem.* any of a group of arginine-rich, strongly basic proteins that are not coagulated by heat, occurring primarily in the sperm of fish. [1870–75; PROT- + AMINE]

prot·a·nom·a·ly (prōt′n om′ə lē), *n. Ophthalm.* a defect of vision characterized by a diminished response of the retina to red. [1935–40; PROT- + ANOMALY] —**prot′a·nom′a·lous,** *adj.*

pro·ta·no·pi·a (prōt′n ō′pē ə), *n. Ophthalm.* a defect of vision in which the retina fails to respond to red or green. [1900–05; < NL; see PROT-, AN-¹, -OPIA] —**pro·ta·nop·ic** (prōt′n op′ik), *adj.*

prot·a·sis (prot′ə sis), *n., pl.* **-ses** (-sēz′). **1.** the clause expressing the condition in a conditional sentence, in English usually beginning with *if.* Cf. **apodosis. 2.** the first part of an ancient drama, in which the characters are introduced and the subject is proposed. Cf. **catastasis, catastrophe** (def. 4), **epitasis. 3.** (in Aristotelian logic) a proposition, esp. one used as a premise in a syllogism. [1610–20; < LL: introduction in a drama < Gk *prótasis* proposition, lit., a stretching forward, equiv. to *pro-* PRO-² + *tásis* a stretching (*ta-,* verbid s. of *teínein* to stretch + *-sis* -SIS)]

pro·te·an (prō′tē ən, prō tē′-), *adj.* **1.** readily assuming different forms or characters; extremely variable. **2.** changeable in shape or form, as an amoeba. **3.** (of an actor or actress) versatile; able to play many kinds of roles. **4.** (*cap.*) of, pertaining to, or suggestive of Proteus. [1590–1600; PROTE(US) + -AN] —**pro′te·an·ism,** *n.*

pro·te·ase (prō′tē ās′, -āz′), *n. Biochem.* any of a group of enzymes that catalyze the hydrolytic degradation of proteins or polypeptides to smaller amino acid polymers. [1900–05; PROTE(IN) + -ASE]

pro·tect (prə tekt′), *v.t.* **1.** to defend or guard from attack, invasion, loss, annoyance, insult, etc.; cover or shield from injury or danger. **2.** *Econ.* to guard (the industry or an industry of a nation) from foreign competition by imposing import duties. **3.** to provide funds for the payment of (a draft, note, etc.). —*v.i.* **4.** to provide, or be capable of providing, protection: *a floor wax that protects as well as shines.* [1520–30; < L *prōtēctus,* ptp. of *prōtegere* to cover in front, equiv. to *prō-* PRO-¹ + *teg-,* s. of *tegere* to cover (akin to TOGA, THATCH) + *-tus* ptp. suffix] —**pro·tect′i·ble, pro·tect′a·ble,** *adj.* —**pro·tect′i·bil′i·ty, pro·tect′a·bil′i·ty,** *n.*

—**Syn. 1.** screen, shelter. See **defend.** —**Ant. 1.** attack.

pro·tect·ant (prə tek′tənt), *n.* a substance, as a chemical spray, that provides protection, as against insects, frost, rust, etc.; protective agent. [1660–70, for an earlier sense; PROTECT + -ANT]

pro·tec·tee (prō′tek tē′, prə tek-), *n.* a person, as a head of state, for whom official protection is provided. [1595–1605; PROTECT + -EE]

pro·tect·ing (prə tek′ting), *adj.* providing protection or shelter. [1620–30; PROTECT + -ING²] —**pro·tect′ing·ly,** *adv.* —**pro·tect′ing·ness,** *n.*

pro·tec·tion (prə tek′shən), *n.* **1.** the act of protecting or the state of being protected; preservation from injury or harm. **2.** a thing, person, or group that protects: *This vaccine is a protection against disease.* **3.** patronage. **4.** *Insurance.* coverage (def. 1). **5.** *Informal.* **a.** money paid to racketeers for a guarantee against threatened violence. **b.** bribe money paid to the police, politicians, or other authorities for overlooking criminal activity. **6.** *Econ.* protectionism. **7.** a document that assures safety from harm, delay, or the like, for the person, persons, or property specified in it. **8.** *Archaic.* a document given by the U.S. customs authorities to a sailor traveling abroad certifying that the holder is a citizen of the U.S. [1275–1325; ME *proteccio(u)n* < LL *prōtēctiōn-* (s. of *prōtēctiō*) a covering in front. See PROTECT, -ION] —**pro·tec′tion·al,** *adj.*

—**Syn. 1.** security, refuge, safety. **2.** guard, defense, shield, bulwark. See **cover. 3.** aegis, sponsorship. **7.** pass, permit.

pro·tec·tion·ism (prə tek′shə niz′əm), *n.* **1.** *Econ.* the theory, practice, or system of fostering or developing domestic industries by protecting them from foreign competition through duties or quotas imposed on importations. **2.** any program, policy, or system of laws that seeks to provide protection for property owners, wildlife,

CONCISE PRONUNCIATION KEY: act, cāpe, dâre, pärt; set, ēqual; if, īce; ox, ōver, ôrder, oil, bo͝ok, bo͞ot, out; up, ûrge; child; sing; shoe; thin, *th*at; zh as in *treasure.* ə = *a* as in *alone, e* as in *system, i* as in *easily, o* as in *gallop, u* as in *circus;* ′ as in *fire* (fī′r), *hour* (ou′r). l and n can serve as syllabic consonants, as in *cradle* (krād′l), and *button* (but′n). See the full key inside the front cover.

dj.	pro′-O·ri·en′tal, *adj., n.*	pro-Pan′a·ma′, *adj.*	pro′pa·tri·ot′ic, *adj.*	pro-Phil′ip·pine′, *adj.*
j.	pro·or′tho·dox′, *adj.*	pro′-Pan·a·ma′ni·an, *adj., n.*	pro·pa′tri·ot·ism, *n.*	pro-Pol′ish, *adj.*
, *adj.*	pro·or′tho·dox′y, *adj.*	pro·pa′pist, *n., adj.*	pro·pa′tron·age, *adj.*	pro·pol′i·tics, *adj.*
lan, *adj., n.*	pro·pac′i·fism, *n.*	pro-Par′a·guay′, *adj.*	pro·pay′ment, *adj.*	pro′-Por·tu·guese′, *adj., n., pl.* **-guese.**
ij.	pro·pac′i·fist, *n., adj.*	pro′-Par·a·guay′an, *adj., n.*	pro′-Pe·ru′vi·an, *adj., n.*	

the environment, etc. [1855–60; PROTECTION + -ISM] —pro·tec/tion·is/tic, adj. —pro·tec/tion·ist, n., adj.

pro·tec·tive (pre tek/tiv), adj. 1. having the quality or function of protecting: a protective covering. 2. tending to protect. 3. Econ. of, pertaining to, or designed to favor protectionism: protective tariffs. 4. defensive (def. 4). [1655–65; PROTECT + -IVE] —pro·tec/tive·ly, adv. —pro·tec/tive·ness, n.

protec/tive col/loid, Physical Chem. a lyophilic colloid added to a lyophobic sol to lessen its sensitivity to the precipitating effect of an electrolyte. [1905–10]

protec/tive colora/tion, coloration or anything likened to it that eliminates or reduces visibility or conspicuousness. [1890–95]

protec/tive custody, detention of a person by the police solely as protection against a possible attack or reprisal by someone. [1935–40]

protec/tive slope/, a slope given to a yard or the like to drain surface water away from a building.

protec/tive sys/tem, Econ. protectionism (def. 1). [1810–20]

pro·tec·tor (pre tek/tar), n. 1. a person or thing that protects; defender; guardian. 2. Eng. Hist. a. a person in charge of the kingdom during the sovereign's minority, incapacity, or absence. b. (cap.) Also called Lord Protector. the title of the head of the government during the period of the Protectorate, held by Oliver Cromwell (1653–58) and by Richard Cromwell, his son (1658–59). [1325–75; < LL (see PROTECT, -TOR); r. ME protectour < MF] —pro·tec/tor·al, adj. —pro·tec/tor·less, adj. —pro·tec/tor·ship/, n.

pro·tec·tor·ate (pre tek/tar it), n. 1. the relation of a strong state toward a weaker state or territory that it protects and partly controls. 2. a state or territory so protected. 3. the office or position, or the term of office, of a protector. 4. the government of a protector. 5. (cap.) Eng. Hist. the period (1653–59) during which Oliver and Richard Cromwell held the title of Lord Protector, sometimes extended to include the period of the restoration of the Rump Parliament (1659–60). [1685–95; PROTECTOR + -ATE³]

pro·tec·to·ry (pre tek/te rē), n., pl. -ries. an institution for the care of destitute or delinquent children. [1650–60; PROTECT + -ORY²]

pro·tect·ress (prō tek/tris), n. a woman who guards or defends someone or something; protector. [1560–70; PROTECT(O)R + -ESS]
—Usage. See -ESS.

pro·té·gé (prō/te zhā/, prō/te zhā/), n. a person under the patronage, protection, or care of someone interested in his or her career or welfare. [1780–90; < F, n. use of ptp. of protéger to protect < L prōtegere. See PROTECT]

pro·té·gée (prō/te zhā/, prō/te zhā/), n. a woman under the patronage, protection, or care of someone interested in her career or welfare. [1770–80; < F, fem. of protégé PROTÉGÉ]

pro·tein (prō/tēn, -tē in), n. 1. Biochem. any of numerous, highly varied organic molecules constituting a large portion of the mass of every life form and necessary in the diet of all animals and other nonphotosynthesizing organisms, composed of 20 or more amino acids linked in a genetically controlled linear sequence into one or more long polypeptide chains, the final shape and other properties of each protein being determined by the side chains of the amino acids and their chemical attachments: proteins include such specialized forms as collagen for supportive tissue, hemoglobin for transport, antibodies for immune defense, and enzymes for metabolism. 2. the plant or animal tissue rich in such molecules, considered as a food source supplying essential amino acids to the body. 3. (formerly) a substance thought to be the essential nitrogenous component of all organic bodies. —adj. 4. Biochem. of the nature of or containing protein. Also, **pro·teid** (prō/tēd, -tē id). [1835–45; < G Protein < Gk prōte(ios) primary + G -in -IN²; r. proteine < F] —pro·tein·a·ceous (prō/tē nā/shas, -tē i nā/-), pro·tein/ic, pro·tei/nous, adj.

pro·tein·ase (prō/tē nās/, -nāz/, -tē i-), n. Biochem. any of a group of enzymes that are capable of hydrolyzing proteins. [1925–30; PROTEIN + -ASE]

pro/tein coat/, Microbiol. capsid.

pro·tein·oid (prō/tē noid/, -tē e-), n. Biochem. a polymer of amino acids resembling a biological polypeptide but formed abiotically: suggested as a possible intermediate in protein development during primitive earth conditions. [1955–60; PROTEIN + -OID]

pro/tein syn/thesis, Biochem. the process by which amino acids are linearly arranged into proteins through the involvement of ribosomal RNA, transfer RNA, messenger RNA, and various enzymes.

pro·tein·u·ri·a (prō/tē nŏŏr/ē e, -nyŏŏr/-, -tē e-), n. [illegible] the presence of abnormally large amounts of [illegible] ting from kidney dis- [illegible] excessive exercise, or [illegible] 15: < NL; see PROTEIN,

rē/), Latin. 1. temporarily; for the time being. 2. temporary.

pro·tend (prō tend/), Archaic. —v.t. 1. to stretch forth. 2. to extend in duration. —v.i. 3. to stretch forward. [1400–50; late ME protenden < L protendere to stretch out, extend, equiv. to pro- PRO-¹ + tendere to stretch; see TEND¹]

pro·ten·sive (prō ten/siv), adj. Archaic. extended in dimension or extended in time. [1635–45; < L protēns(us) (ptp. of protendere to PROTEND) + -IVE, on the model of EXTENSIVE, INTENSIVE, etc.] —pro·ten/sive·ly, adv.

pro·te·o·gly·can (prō/tē ō glī/kan), n. Biochem. a macromolecule composed of a polysaccharide joined to a polypeptide and forming the ground substance of connective tissue. [1969; PROTE(IN) + -O- + GLYC- + -an, var. of -ANE]

pro·te·ol·y·sis (prō/tē ol/e sis), n. Biochem. the breaking down of proteins into simpler compounds, as in digestion. [1875–80; proteo- (comb. form repr. PROTEIN) + -LYSIS] —pro·te·o·lyt·ic (prō/tē e lit/ik), adj.

pro·te·ose (prō/tē ōs/), n. Biochem. any of a class of soluble compounds derived from proteins by the action of the gastric juices, pancreatic juices, etc. [1885–90; PROTE(IN) + -OSE²]

protero-, a combining form meaning "earlier," "before," "former," used in the formation of compound words: proterotype. Also, esp. before a vowel, **proter-.** Cf. **proto-.** [< Gk, comb. form repr. próteros, comp. formed from pró; see PRO-²]

prot·er·o·type (prot/ar e tīp/, prō/tar-), n. a primary type. [PROTERO- + TYPE]

Prot·er·o·zo·ic (prot/ar e zō/ik, prō/tar-), Geol. —adj. 1. noting or pertaining to the latter half of the Precambrian Era, from about 2.5 billion to 570 million years ago, characterized by the appearance of bacteria and marine algae; Algonkian. —n. 2. the Proterozoic division of geologic time or the rock systems formed then; Algonkian. See table under **geologic time.** [1905–10; PROTERO- + ZO- + -IC]

pro·test (n. prō/test; v. pre test/, prō/test), n. 1. an expression or declaration of objection, disapproval, or dissent, often in opposition to something a person is powerless to prevent or avoid: a protest against increased taxation. 2. Com. a. a formal notarial certificate attesting the fact that a check, note, or bill of exchange has been presented for acceptance or payment and that it has been refused. b. the action taken to fix the liability for a dishonored bill of exchange or note. 3. Law. a. (upon one's payment of a tax or other state or city exaction) a formal statement disputing the legality of the demand. b. a written and attested declaration made by the master of a ship stating the circumstances under which some damage has happened to the ship or cargo, or other circumstances involving the liability of the officers, crew, etc. 4. Sports. a formal objection or complaint made to an official. —v.i. 5. to give manifest expression to objection or disapproval; remonstrate. 6. to make solemn or earnest declaration. —v.t. 7. to make a protest or remonstrance against; object to. 8. to say in protest or remonstrance. 9. to declare solemnly or earnestly; affirm; assert. 10. to make a formal declaration of the nonacceptance or nonpayment of (a bill of exchange or note). 11. Obs. to call to witness. [1350–1400; (n.) ME < MF (F protêt), deriv. of protester to protest < L prōtestārī to declare publicly, equiv. to prō- PRO-¹ + testārī to testify, deriv. of testis a witness; (v.) late ME protesten < MF protester] —pro·test/a·ble, adj. —pro·test/er, pro·tes/tor, n. —pro·test/ing·ly, adv. —pro·test/ive, adj.
—Syn. 5. complain. 6. asseverate, avow, aver, attest. See **declare.** —Ant. 1. approval. 5. approve.

Prot·es·tant (prot/e stant or, for 4, 6, pre tes/tant), n. 1. any Western Christian who is not an adherent of a Catholic, Anglican, or Eastern church. 2. an adherent of any of those Christian bodies that separated from the Church of Rome during the Reformation, or of any group descended from them. 3. (originally) any of the German princes who protested against the decision of the Diet of Speyer in 1529, which had denounced the Reformation. 4. (l.c.) a person who protests. —adj. 5. belonging or pertaining to Protestants or their religion. 6. (l.c.) protesting. [1530–40; < G or F, for L prōtestantēs, pl. of prp. of prōtestārī to bear public witness. See PROTEST, -ANT]

Prot/estant Epis/copal Church/. See **Episcopal Church in America.**

Prot/estant eth/ic. See **work ethic.** Also called **Prot/estant work/ eth/ic.** [1925–30]

Prot·es·tant·ism (prot/e stan tiz/em), n. 1. the religion of Protestants. 2. the Protestant churches collectively. 3. adherence to Protestant principles. [1640–50; PROTESTANT + -ISM]

Prot·es·tant·ize (prot/e stan tīz/), v.t., -ized, -iz·ing. to convert or cause to conform to Protestantism. Also, esp. Brit., **Prot/es·tant·ise/.** [1825–35; PROTESTANT + -IZE]

Prot/estant Reforma/tion, reformation (def. 2).

prot·es·ta·tion (prot/e stā/shen, prō/te-, -te-), n. 1. the act of protesting or affirming. 2. a solemn or earnest formal expression or

Pro·te·us (prō/tē es, -t[illegible] god, son of Oceanus and [illegible] assume different forms [illegible] thing that readily change[illegible] ples, etc. 3. (l.c.) Bacter[illegible] aerobic bacteria of the g[illegible] as pathogens in the gas[illegible] tracts of humans.

pro·tha·la·mi·on (pr[illegible] -mi·a (-mē e). a song [illegible] marriage. [1597; PRO-² [illegible] mund Spenser]

pro·tha·la·mi·um (pr[illegible] (-mē e). prothalamion.

pro·thal·li·um (prō [illegible] (-thal/ē e). 1. Bot. the [illegible] plants. 2. the analogo[illegible] seed-bearing plants. [1[illegible] thallion, dim. of thallo[illegible] thal/li·al, pro·thal/lic, [illegible] adj. —pro·thal/loid, a[illegible]

pro·thal·lus (prō thal[illegible] prothallium. [1850–55;

proth·e·sis (proth/e s[illegible] 1. the addition of a sou[illegible] a word, as in Spanish [illegible] 2. Eastern Ch. a. Also [illegible] tion and preliminary [illegible] ments. b. the table on [illegible] the sanctuary or bema [illegible] cap.) Gk. Antiq. a rep[illegible] in state. [1665–75; < [illegible] fore. See PRO-², THESIS [illegible] —pro·thet/i·cal·ly, a[illegible]

pro·thon·o·tar·y (p[illegible] n., pl. -tar·ies. 1. a [illegible] courts of law. 2. Ro[illegible] members of the col[illegible] charged chiefly with [illegible] canonizations. b. an [illegible] prelates. 3. Gk. Orth[illegible] triarch of Constantin[illegible] late ME < ML proth[illegible] prōtonotários. See PR[illegible] al (prō thon/e târ/ē [illegible]

prothon/otary p[illegible] ostolic. a member [illegible] Roman Curia. [1545[illegible]

prothon/otary wa[illegible] taria citrea, of the co[illegible] head and underpart[illegible] [1780–90, Amer.; so [illegible] bles the robes tradit[illegible]

prothorac/ic glan[illegible] glands in the anteric[illegible] to promote the seri[illegible] hood. [1885–90]

pro·tho·rax (prō t[illegible] -tho·ra·ces (-thôr/[illegible] of the thorax of an [illegible] [1820–30; < NL; s[illegible] (prō/thō ras/ik, -th[illegible]

pro·throm·bin (p[illegible] protein involved in [illegible] by factors in the p[illegible] called **thromboge[illegible]**

pro·tist (prō/tist). [illegible] isms, classified in [illegible] free-living or aggr[illegible] have diverse repro[illegible] ing the protozoan[illegible] some classificatio[illegible] the more primitive [illegible] distribute the org[illegible] and Animalia ac[illegible] [1885–90; < NL P[illegible] < Gk prṓtistos ([illegible] prōtos first; see P[illegible] n. —pro·tis/tic.

Pro·tis·ta (prō t[illegible] a taxonomic king[illegible] < NL; see PROTIS[illegible]

pro·tis·tol·o·gy [illegible] Protista. [1910–1[illegible] to·log·i·cal (prō [illegible] gist, n.

pro·ti·um (prō/[illegible] and most comm[illegible] [1930–35; PROT- [illegible]

proto-, a combi[illegible] "earliest form o[illegible] words (protomar[illegible] in chemical term[illegible] compounds, or t[illegible] of an element. [illegible] comb. form repr[illegible] see PRO²-]

pro·to·ac·tin·i[illegible] protactinium.

APPENDIX II

Copy of Reference

Sara et al. (Sara 2)

The Biological Role of Truncated Insulin-like Growth Factor-1

and the Tripeptide GPE in the Central Nervous System

The Biological Role of Truncated Insulin-like Growth Factor-1 and the Tripeptide GPE in the Central Nervous System[a]

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INTRODUCTION

Since early in this century, attempts have been made to identify substances present in serum and organ extracts that are capable of promoting the growth of the nervous system. Today several such growth-promoting factors have been isolated and identified, such as nerve growth factor (NGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and insulin-like growth factors (IGFs). It has become clear that these growth factors are endogenously produced within the developing nervous system at specific growth phases and that they interact to regulate the growth and development of the central nervous system (CNS). A role for the IGFs in the regulation of CNS growth was first implicated 20 years ago by the finding that growth hormone had an indirect action on brain growth which was believed to be mediated by the production of a brain growth factor from either the placenta or the fetus.[1,2]

[a]These studies have been supported by the Swedish Medical Research Council, Oskar Fund, Swedish Cancer Foundation, and the Cancer Fund in Stockholm.

TRUNCATED IGF-1 AND GPE

A bioassay to determine fetal brain cell DNA synthesis[3] and later a radioreceptor assay using fetal brain plasma membranes[4] were developed to isolate the brain growth factor from human fetal brain tissue. Partial amino acid sequencing revealed the brain growth factor to be identical to IGF-1 over the first 29 amino acids, but to have a truncated aminoterminus lacking the first three amino acids of IGF-1 (FIGURE 1).[5] The carboxyl terminal amino acid was shown to be identical to that of IGF-1. An identical truncated IGF-1 was subsequently isolated from the adult human brain.[6] Intact IGF-1 could not be detected in either the fetal or the adult human brain. The formation of truncated IGF-1 or -3N-IGF-1 appeared to be specific to the tissue extract since only intact IGF-1 was isolated from serum when the same purification process was used.

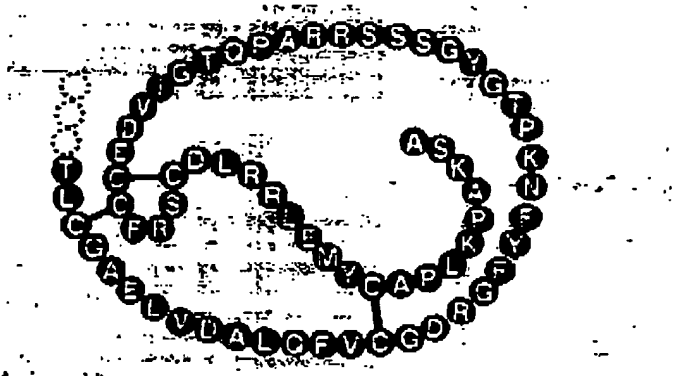

FIGURE 1. Amino acid sequence of human truncated IGF-1 (-3N-IGF-1). The single letter amino acid code is used. The protein lacks the aminoterminal tripeptide GPE. Truncated IGF-1 has been identified in human brain, human platelets, porcine uterus, and bovine colostrum.

The possibility of further structural modifications in the brain IGF could not be discounted at that time since the purified peptide displayed greatly enhanced neurotrophic activity compared to IGF-1. The complete amino acid sequence of the peptide has now been deduced from the nucleotide sequence of human fetal brain IGF-1 cDNA.[7] Using reverse transcriptase polymerase chain reaction (RT-PCR) to amplify cDNA obtained from isolated human fetal brain, two cDNA sequences encoding precursor proteins that correspond to IGF-1a and IGF-1b were obtained. Thus the amino terminal truncation of the IGF-1 protein represents the only sequence difference in the brain IGF-1 (FIGURE 1). The brain truncated IGF-1 most likely arises from posttranslational modification of the IGF-1 precursor protein. As yet it is unclear as to whether the precursor to truncated IGF-1 is the a or b form of the IGF-1 prohormone. Recently, the amino terminal tripeptide of IGF-1, namely, glycyl-prolyl-glutamate (GPE), has been identified in human brain. Thus there are two protein products from expression of the IGF-1 gene in the human brain, namely, truncated IGF-1 and the tripeptide GPE.

The presence of IGF-1 in the nervous system appears to be a phylogenetically ancient phenomenon. Using immunological methods, IGF-1 has been localized in the nervous system as well as the gut of lower vertebrates, including bony and cartilaginous fish and cyclostomi, as well as protochordates. For example, IGF-1 immunoreactive perikarya and fibers have been observed in all levels of the brain of the Atlantic hagfish, *Myxine glutinosa*.[8] IGF-1-like immunoreactivity has also been localized in central neurones of the urochordate *Ciona intestinalis* and the cephalochordate *Branchiostoma lanceolatum*.[9] Thus the presence of IGF-1 in the "brain-gut axis" has been well preserved during vertebrate evolution. The identity of the IGF-1-like molecule in the brain-gut axis of the lower vertebrates and protochordates remains to be determined. The nucleotide sequence of an IGF cDNA isolated from *Myxine glutinosa* showed 70% homology to the A and B domains of both human IGF-1 and IGF-2,[10] and a hybrid insulin/IGF cDNA related to both human insulin and IGFs has been cloned from *Branchiostoma californiensis*.[11] Chan *et al.* have proposed that the latter hybrid molecule represents the transitional form prior to insulin and IGF divergence at an early stage of vertebrate evolution.[11] It is of interest that the deduced amino acid sequence of the hybrid insulin/IGF molecule reveals a different aminoterminal dipeptide compared to mammalian IGF-1. A plasma membrane receptor similar to that of mammalian IGF-1 receptor has also been identified in the nervous system of lower vertebrates, including *Myxine glutinosa*.[12]

The truncated IGF-1 has been identified in several tissues (FIGURE 1). Ogasawara *et al.* identified truncated IGF-1 in porcine uterus where the peptide accounted for the complete mitogenic activity of uterine extracts.[13] Truncated IGF-1 has also been isolated from human platelets.[14] Lysates of human platelets contain intact as well as truncated IGF-1 and IGF-2. IGF-1 was released from the platelets during degranulation, suggesting a role in wound healing.[15] Francis *et al.* have identified truncated IGF-1 in bovine colostrum where intact IGF-1 was additionally found to be present.[16] In all studies during its purification, truncated IGF-1 displayed enhanced biological activity. With the availability of synthetic and recombinant truncated IGF-1, the reason for this enhanced biological potency became apparent. Truncated IGF-1 binds only weakly to the IGF binding proteins (IGFBPs).[17–20] Although truncated IGF-1 shows some binding to IGFBP-3, a marked reduction in binding affinity to IGFBP-1, -2, -6 has been found in comparison to intact IGF-1 (less than 1%). Analogues of IGF-1 with substitutions in the aminoterminus pentapeptide have identified Glu in residue 3 as playing a significant role in IGFBP binding. In a wide variety of cultured cells, it has been demonstrated that the enhanced biological activity of the truncated IGF-1 is most likely due to its failure to be bound by IGFBPs which can compete with the IGF-1 receptor and attenuate the biological activity of IGF-1. Thus the failure to bind to IGFBPs results in a greater availability of truncated IGF-1 to the target cell receptors (FIGURE 2).

BIOLOGICAL ACTION IN CNS

Truncated IGF-1 (-3N:IGF-1) and the tripeptide GPE display separate biological functions in the nervous system which are mediated via distinct receptors on their target cells. Truncated IGF-1 has a potent neurotrophic action via interaction in the IGF-1 receptor in the CNS. The IGF-1 receptor is widely distributed throughout the CNS,[21] and its expression is enhanced during the rapid growth phase of early life.[22] IGF-1's neurotrophic action predominates during early development, when the IGFs regulate the growth and differentiation of the nervous system. *In vitro* studies have demonstrated that IGF-1 stimulates the proliferation of neuroblast and glioblast

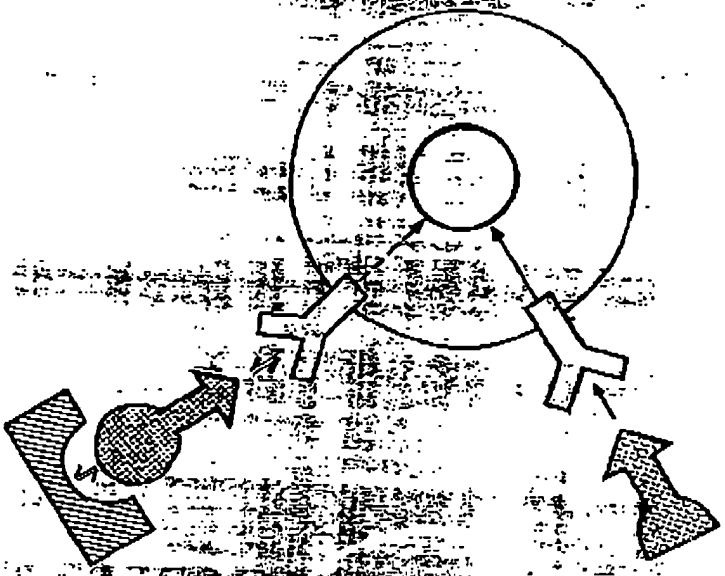

FIGURE 2. Model to account for the enhanced neurotrophic activity of truncated IGF-1 compared to intact IGF-1. Failure to bind appreciably with the IGFBPs is hypothesized to result in greater availability of truncated IGF-1 to the IGF-1 receptors on target cells.

precursor cells, as well as their differentiation.[23] Several studies indicate that IGF-1 may play an important role in synapse formation and myelination.[24-27] Comparison between the various IGFs reveals that truncated IGF-1 displays a far greater neurotrophic activity compared to intact IGF-1 or IGF-2. Using fetal brain cells *in vitro*, Carlsson-Skwirut *et al.* showed that this enhanced neurotrophic potency was due to failure of truncated IGF-1 to bind to binding proteins.[28] The addition of IGFBP-1 to the culture medium could inhibit the stimulation of fetal brain cell DNA synthesis by intact IGF-1 but had no effect on the action of truncated IGF-1. A similar action has been observed with intraocular transplantation of brain tissue by Giacobini *et al.*[29] Truncated IGF-1 had a potent growth-promoting action on parietal cortex and spinal cord grafts transplanted to the anterior chamber of the eye of adult rats. This *in vivo* model allows for direct observation of graft survival and growth. The action of truncated IGF-1 was developmentally as well as regionally specific. For example, a significant growth-promoting action was observed in spinal cord grafts at embryonic day E14 but not at E16. It was suggested that these differences reflected the stage of receptor maturation and function in the various regions. This is in accordance with the expression of the IGF-1 gene in specific cell populations at discrete times during CNS development.[2,3] Intact IGF-1 failed to stimulate the growth of brain transplants, presumably due to the presence of IGFBPs which were present in the vitreous fluid.

Similarly, when administered intravenously, truncated IGF-1 displays reduced binding to IGFBPs in the circulation, and the truncated form is more rapidly cleared

from the blood[32] and is also degraded faster than intact IGF-1.[31] Consequently, the acute hypoglycemic effect of truncated IGF-1 is greater than that of intact IGF-1.[3] Increased degradation due to low association with IGFBPs most likely explains the failure to observe any significant enhancement in growth following the subcutaneous administration of truncated IGF-1 to neonatal rats in spite of enhancement being observed following intact IGF-1 administration. In contrast to the growth of normal rats, enhanced growth has been observed in growth-hormone-deficient lit/lit mouse following truncated IGF-1 administration.[33] Truncated IGF-1 has similarly been reported to be more potent than intact IGF-1 in regulating nitrogen balance and muscle protein metabolism in nitrogen-restricted rats.[34]

The aminoterminal tripeptide of IGF-1, GPE, displays a different range of biological actions compared to truncated IGF-1.[35] These effects are not mediated by IGF receptors. As shown in FIGURE 3, GPE fails to cross-react in the IGF-1 receptor and does not influence the binding of either intact or truncated IGF-1 to the receptor. The tripeptide similarly fails to cross-react in the IGF-2 receptor. GPE does not bind to IGFBPs nor does it influence the association of the IGFs to their binding proteins. Extensive *in vivo* studies have not revealed any growth-promoting activity of GPE. The results of one such study are summarized in FIGURE 4. Growth was followed in rats receiving 30 μg GPE subcutaneously (sc) per day from days 3 to 15 of postnatal life. As shown in FIGURE 4, no significant growth effects, including tail length and organ weights, were observed. However at maturity, the animals receiving GPE during this preweaning period displayed a significant increase in activity measurements in an open field test. It has since been demonstrated that GPE plays a neuromodulatory role in the CNS,[36] which may account for the changes in activity observed in the GPE-treated rats.

The structure of GPE suggested that it may interact in receptors for glutamate which is a major excitatory amino acid neurotransmitter in the CNS. Using rat synaptic membranes, it was shown that GPE cross-reacted in the *N*-methyl-D-

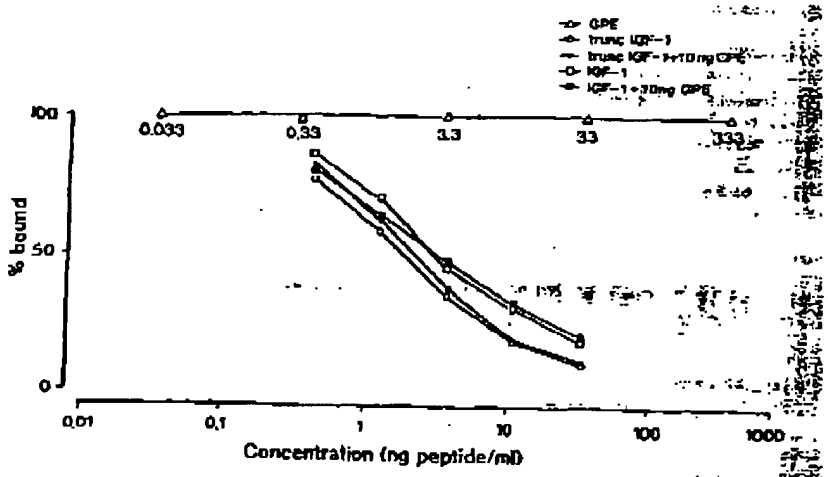

FIGURE 3. Competition with ^{125}I-IGF-1 for binding to human fetal brain membranes. Data are expressed as the percentage bound in the absence of competing peptide.

aspartate (NMDA) but not the kainate or quisqualate type of glutamate receptor.[39] While the carboxyl terminal glutamate was necessary for NMDA receptor binding, the aminoterminal glycine potentiated this interaction. Glycine has earlier been shown to potentiate responses mediated via the NMDA receptor and has been suggested to be a specific regulator of the NMDA receptor via binding to an allosteric site.[36] GPE facilitates the release of dopamine from cortical slices. As shown by the use of a selective competitive antagonist, this action is mediated via interaction in the NMDA receptor.[5] In addition, GPE has a potent stimulatory action on acetylcholine release from cortical neurones.[35,37] This action cannot be inhibited by NMDA receptor blocking agents, and acetylcholine potentiation is mediated via an as yet unidentified receptor. GPE does not interact with choline uptake sites or muscarinic receptors on neurones. Although GPE interacts in nicotinic binding sites in the rat cortex, this interaction occurs at a concentration several orders of magnitude greater than that required to potentiate acetylcholine release. Thus the receptor mechanism for acetylcholine potentiation remains to be identified. It had been reported earlier that intact IGF-1 potentiates acetylcholine release from cortical slices.[38] Similarly, IGF-1 has been reported to enhance catecholamine release from chromaffin cells,[39] while truncated IGF-1 fails to enhance acetylcholine release from the cortical slices.[38] It may be suggested that the action of intact IGF-1 on neurotransmitter release was due to the tripeptide GPE. However, during this acute experiment, significantly less truncated IGF-1 was taken up by the cortical slices during the 30-minute incubation period. Thus, in the adult cortex, formation of the IGFBP complex may be necessary for transport across the microvascular barrier to directly interact with neurones and induce an acute action.

Neuronal activity can be modulated by GPE. This has been demonstrated in single cortical neurones following iontophoretic application of GPE. As shown in FIGURE 5, GPE alone has no effect on the electrophysiological activity of the neurone; however, when applied together with glutamate, it potentiates the action of

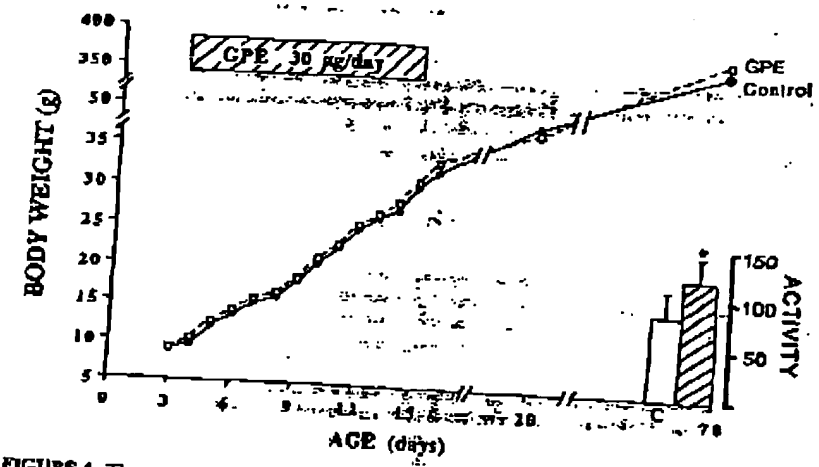

FIGURE 4. The growth of rats receiving either 30 µg GPE sc/day or vehicle alone from days 3 to 15 of postnatal life. No significant effect on body weight was observed. At 70 days of age, open field behavior was examined. GPE-treated rats displayed a significant increase in activity scores.

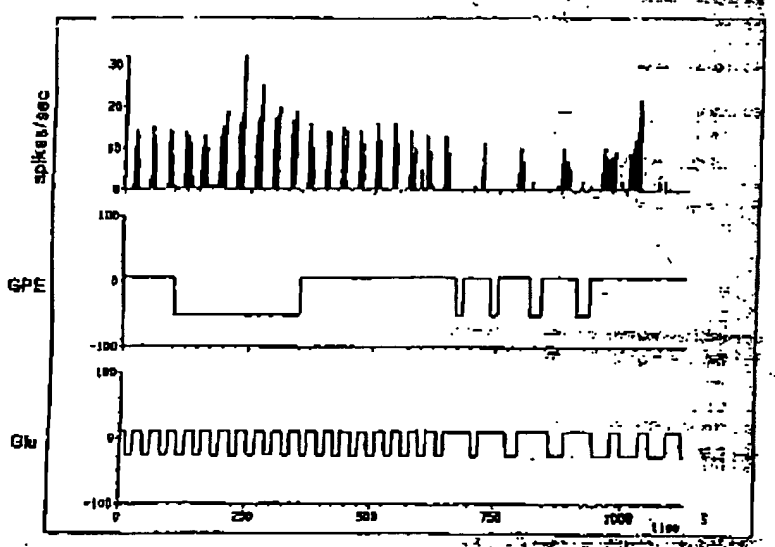

FIGURE 5. Cortical neurone electrophysiological activity following microiontophoretic app cation of GPE. The effect of GPE on spontaneous as well as glutamate-driven single cell activi determined as spikes/second is shown.

the glutamate-driven neurone. Similarly, in the spinal cord, GPE has no direc influence on motor neurone activity when applied intrathecally; however GP potentiates the facilitated spinal cord reflex in response to other stimuli.

CONCLUSION

Thus there are at least two protein products from expression of the IGF-1 gene the CNS. These proteins result from posttranslational modification of the IGF- precursor protein. Truncated IGF-1 (-3N:IGF-1) acts as a potent neurotrophi factor and this action is mediated via the IGF-1 receptor. The tripeptide -GPI appears to have a quite different CNS function, namely, the modulation of neurotrans mitter release. This potentiating action is mediated at least in part via interaction w the NMDA receptor. This is the first example of a product from a growth factor gen being involved in neurotransmission in the CNS. A similarly novel role for th neurotransmitter acetylcholine has been suggested in the regulation of brain growth.

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APPENDIX III

Copy of Reference

Sara et al. (Sara 3)

Neuroactive Products of IGF-1 and IGF-2 Gene Expression in the CNS

NEUROACTIVE PRODUCTS OF IGF-I AND IGF-2 GENE EXPRESSION IN THE CNS

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INTRODUCTION

The insulin-like growth factors (IGFs) consist of IGF-1 and IGF-2, as well as variants arising from either alternative RNA processing or post-translational modification of the IGF precursor. In the extracellular fluid or circulating, the IGFs are associated with their carrier proteins which are believed to function as transporters, directing the IGFs to their target cells (35). The IGFs act as both endocrine hormones on distal target cells, as well as locally as paracrine or autocrine hormones. While the IGFs have long been recognized as growth and anabolic factors for a wide variety of tissues and cell types, such as cartilage, muscle, and fibroblasts, their role within the nervous system has only been widely recognized over the last several years. However, historically this can be traced back to the experiments of Stephan Zamenhof in the 1940's, who demonstrated that crude pituitary extracts of growth hormone stimulated the growth of tadpole and rat brains. This brain growth-promoting activity was later shown to be due to a growth hormone dependent growth factor, later identified as truncated IGF-1 (32). Both IGF-1 and IGF-2 are synthesized within the central nervous system where they are believed to fulfill different functions mediated via their receptors.

BIOSYNTHESIS OF IGF-1 IN THE CNS

IGF-1 Gene Expression - Characterization, Localization and Regulation

The IGF-1 gene is expressed within the CNS in a developmentally and regionally specific manner. This has been demonstrated in both rats (30) and man (31) where IGF-1 mRNA is far more abundant during fetal life than in the adult. In the adult, regional specificity has only been examined extensively in the rat, where the major expression was found in the olfactory bulb and spinal cord (30).

The primary transcript from the IGF-1 gene can be alternatively spliced to result in either IGF-1a or IGF-1b mRNA which encode prohormones differing in the length and structure of their carboxylterminal E domains (29). The IGF-1 gene transcripts have recently been characterized in the human brain. Using PCR

et al (in preparation) have obtained the nucleotide sequence of both IGF-1a and IGF-1b cDNA in human fetal brain. The nucleotide sequences of the brain IGF-1a and IGF-1b cDNAs were identical to that obtained in other tissues, such as human liver, with the exception of a base change in position 270 of IGF-1a cDNA. Although this base change may have arisen from the techniques employed, it was repeatedly found using either cloning or direct sequencing. Thus, the possibility of a mutation in this position, which does not influence the amino acid encoded, may be considered. Both IGF-1a and IGF-1b mRNA have been identified in the rat brain by solution hybridization/RNAse protection assay (22). However, unlike in man where the presence of exon 4 or 5 is mutually exclusive, both are present in the IGF-1b mRNA of the rat and also the mouse, which leads to a change in the translational reading frame (39). Thus, the carboxyterminal peptides of the IGF-1b in murine and human vary considerably. In addition, in both the mouse and the rat, transcription appears to be initiated at different sites in the IGF-1 gene. The expression of these 5' untranslated regions is tissue specific, with the class C 5' untranslated region predominating in rat brain (23).

The IGF-1 gene is expressed by both isolated neuronal and glial cells in culture (30). While IGF-1 mRNA has been identified in preparations from whole brain and even various CNS regions, it has only recently been possible, using *in situ* hybridization histochemical techniques, to localize the sites of IGF-1 synthesis within the CNS. In certain areas of the embryonic rat brain, such as the cortex, thalamus, striatum and tectum, the expression of the IGF-1 gene is low and widespread (3). In other areas it appears to be expressed in specific restricted cell groups in a tightly regulated developmental manner, suggesting a specific function during development. In the adult rat brain, IGF-1 mRNA is found in the olfactory bulb, hippocampus and cerebellum (43). As in the embryonic rat (3), intense IGF-1 hybridization in the olfactory bulb is restricted to the glomerular and mitral cells. In the hippocampus, hybridization was to pyramidal cells of Ammon's horn in CA1 and CA2 layers and dentate gyrus, whereas in the cerebellum, it was located to the granular cell layer. These sites of IGF-1 synthesis are adjacent to, or overlap, IGF-1 receptors (42).

The regulation of IGF-1 gene expression within the CNS is poorly understood. In contrast to many other tissues and cells, particularly in the adult where GH is a major stimulator o IGF-1 gene transcription (23), GH does not appear to directly stimulate neuronal or glial cell IGF-1 production. Similarly to the peripheral nervous system (13), IGF-1 synthesis may respond to local tissue injury, however, the signal eliciting this response remains elusive at this stage. Glucocorticoids which are well established inhibitors of brain cell proliferation, have been demonstrated to reduce IGF-1 mRNA in primary cultures of both neuronal and glial cells (1).

Protein Products

The protein products of expression of the IGF-1 gene in the human brain have been isolated and their amino acid sequences determined. Post-translational processing of the IGF-1 prohormone results in two peptides which are proposed to fulfill distinct functions within the CNS (Fig. 1). A truncated form of IGF-1, which lacks the aminoterminal tri-peptide GPE (gly-pro-glu), has been characterized in both fetal and adult human brain (7,33). The truncated IGF-1 appears to be the major gene product in the CNS since no evidence for the presence of intact IGF-1 could be obtained. The truncated IGF-1 similarly appears as the major peptide in bovine colostrum (11), human platelets (19), porcine uterus (26) and has been proposed to represent the locally acting autocrine or paracrine form of IGF-1 (35). The nucleotide sequence of human fetal brain cDNA confirms that the aminoterminal truncation represents the only sequence modification and suggests that this cleavage occurs as a post-translational modification of the IGF-1 prohormone. The second product of proteolytic cleavage of the IGF-1 prohormone is the tripeptide, GPE (32). Although both these products have now been identified within the human brain, the

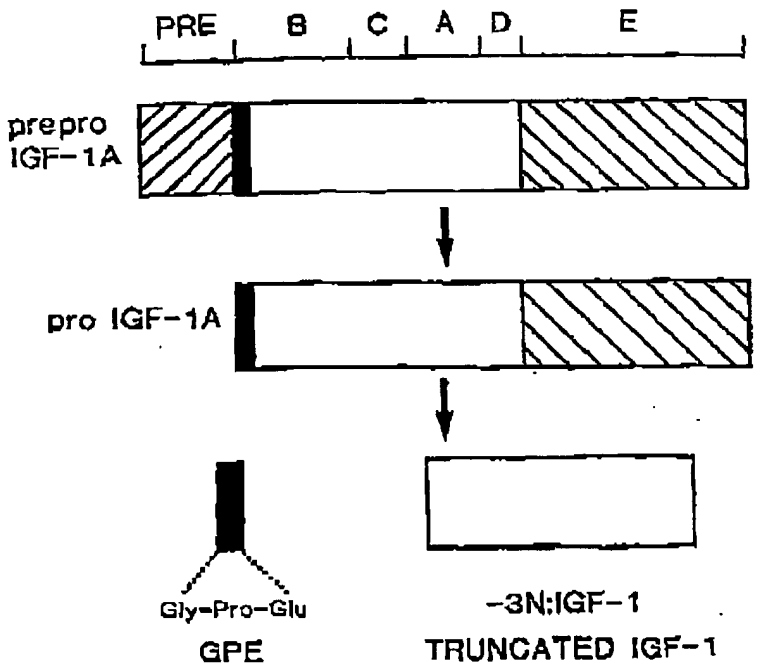

Fig. 1 GPE and truncated IGF-1 are two identified neuroactive products of IGF-1 gene expression in the CNS. These peptides result from post-translational processing of the IGF-1 prohormone, which in the CNS, is proposed to be pro-IGF-1a.

biosynthetic pathway and stages [illegible] completely defined. As illustrated in Figure 1, the predominance of the IGF-1a mRNA in the rat brain has led to the suggestion that proteolytic processing of the IGF-1a prohormone results in the production of truncated IGF-1 and GPE (32, 35).

BIOLOGICAL ACTIVITY

The peptide products from expression of th IGF-1 gene in the brain, namely truncated IGF-1 and GPE, appear to induce biological responses via two separate mechanisms. The action of truncated IGF-1 is mediated via the IGF-1 receptor. GPE does not cross-react in the IGF-1 receptor, but rather in the NMDA receptor, and possibly an additional, as yet undefined, mechanism (34).

The IGF-1 receptor appears to be present on both neurones and glial cells *in vitro*, with a structural subtype displaying altered glycosylation of the hormone-binding a-subunit being present on neurones (6). The expression of the receptor is enhanced during rapid growth phases (36,43). In the adult, the receptor is widely distributed. In the human brain for example, the highest densities of IGF-1 receptor are found in the hippocampus, amygdala and parahippocampal gyrus, followed by cerebellum, cerebral cortex and caudate nucleus (2). The developmental and regional expression of the IGF-1 receptor suggests a role in growth regulation during early development, as well as metabolic regulation in the adult as the distribution in the adult brain occurs in areas of high metabolic activity. Additionally, the presence of both IGF-1 mRNA and immunoreactivity is found to coincide with the distribution and occurrence of the receptors, supporting a paracrine or autocrine role for IGF-1 within the CNS (42).

Over the last decade much evidence has accumulated to demonstrate that IGF-1 and also IGF-2 have a potent growth-promoting action of neuronal and glial cell precursors *in vitro*. Additionally, a role in differentiation has been suggested. For example, IGF-1 has been reported to induce the differentiation of oligodendrocytes from their bipotential precursors (25). The growth-promoting actions of both IGF-1 and IGF-2 appear to be mediated via interaction in the IGF-1 receptor where both peptides cross-react almost equipotently. The biological actions of the IGFs in the CNS have been recently reviewed and will not be detailed here (32). Truncated IGF-1 displays enhanced neurotrophic activity both *in vitro* and *in vivo*, when compared to intact IGF-1 and IGF-2. Enhanced biological activity *in vitro* can be mainly attributed to failure to bind to the IGF-BPs which regulate IGF bioavailability to the target cells (8). Whereas the addition of BP1 to the incubation medium, blocks the action of intact IGF-1, it fails to bind truncated IGF-1 and has no influence on its stimulation of neuronal and glial cell proliferation (8). Failure to be bound by the IGF BPs results in rapid degradation and shorter half-life of truncated IGF-1 in the circulation (10). Thus, systemic administration of truncated IGF-1, as opposed to intact IGF-1, fails to induce a significant growth response in neonatal rats. However, the reverse is found following local application where truncated and not intact IGF-1 displays potent growth-promoting activity. For example, Giacobini et al (12), investigated the effects of both intact and truncated IGF-1 on intraocular grafts of embryonic brain tissue. Truncated IGF-1 displayed a potent neurotrophic action on cortex and spinal cord grafts, whereas intact IGF-1 had no significant effect, presumably due to its binding to BPs present in synovial fluid which prevented bioavailability to the target cells.

GPE is an additional protein product from expression of the IGF-1 gene within the CNS. GPE fails to cross-react in any IGF receptor and does not display growth-promoting activity either *in vitro* or *in vitro* (34). Instead, GPE cross-reacts in the NMDA (N-methyl-D-aspartate) receptor which is a subtype of receptors for the major excitatory amino acid neurotransmitter glutamate (34). GPE interaction appears to be specific for the NMDA receptor subtype as the tripeptide fails to cross-react in either the kainate or quisqualate receptors. The carboxyterminal glutamate residue of GPE is necessary for NMDA receptor binding while the aminoterminal

glycine residue potentiates this binding, suggesting the model shown in Fig. 2. GPE is proposed to cross-react in both the glutamate recognition site as well as the glycine allosteric site of the NMDA receptor. GPE potentiates the release of dopamine via interaction in the NMDA receptor. However, GPE has an additional action which is not mediated via the NMDA receptor, namely the facilitation of acetylcholine release. GPE potentiates the potassium evoked release of acetylcholine from rat striatal slices at concentrations far less than those interacting in the NMDA receptor and this action cannot be inhibited by the use of specific NMDA receptor blockers (34). The mechanism for GPE's potent facilitation of ACh release has not yet been clarified.

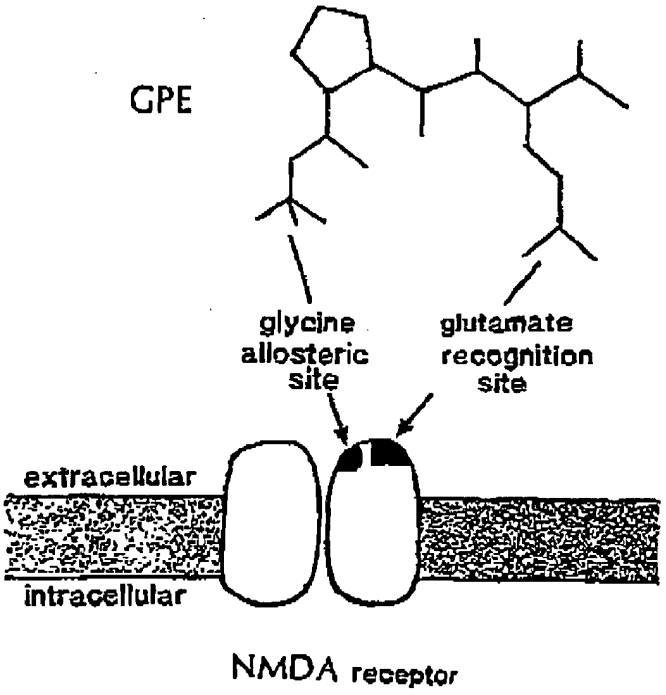

Figure 2. Model of GPEs interaction in the NMDA receptor. It is proposed that GPE cross-reacts in the glycine allosteric site as well as the glutamate recognition site.

In conclusion, there are two identified neuroactive products of IGF-1 gene expression in the CNS, namely truncated IGF-1 and the tripeptide, GPE. Based upon evidence available today, these peptides appear to fulfill quite distinct functions within the CNS. Truncated IGF-1 is proposed to function as an autocrine or paracrine anabolic factor, involved in regulation of proliferation and differentiation and possibly also metabolic regulation in th adult, whereas GPE is believed to act as a neuromodulator regulating neurotransmission. GPE is the first example of the product of a growth factor gene having a specific role in neurotransmission.

IGF-1 Gene Expression - Characterization, Localization and Regulation

The expression of the IGF-2 gene in the nervous system has been well characterized, especially in the rat where the gene continues to be expressed in adult brain (5,24,30). Multiple IGF-2 transcripts, which contain identical coding regions but differ in their untranslated regions, are produced by the initiation of transcription at several different promoter sites in the IGF-2 gene (39). The 4.0kb IGF-2 transcript is greatest in the rat brain, suggesting initiation of transcription at the third promoter site. IGF-2 mRNA is most abundant in the brain during early development, but in contrast to most other tissues in the rat, is also found to be widely distributed in various brain regions in the adult. The explanation for the widespread occurrence of IGF-2 mRNA in brain extracts has become clear with its localization by *in situ* hybridization. The IGF-2 gene is not expressed in neurones or glia but rather in the meninges, choroid plexus, as well as mesenchymal cells surrounding the blood vessels in the adult brain (16,38). Contamination of brain regions by these cells is thus, unavoidable. Similarly in the fetus, apart from the choroid plexus and leptomeninges, IGF-2 mRNA has been detected to be present in a developmentally dependant way in hypothalamus, the floor of the third ventricle, pineal primordium and the pars intermedia of the pituitary (3,4,37). Thus, IGF-2 is synthesized at highly vascularized sites within the CNS, suggesting a role in the production of extracellular fluids and supply of substrates to neural tissue. However, an IGF BP is also synthesized in the choroid plexus (40) and the possibility must also be considered that IGF-2 associates with its BP and is transported to interact in distal IGF-2 receptors throughout the brain.

In the human brain, IGF-2 mRNA is most abundant in the fetus and barely detectable in adult tissue with little membrane contamination (31). A single 6.0kb transcript is found in the fetal brain (31) and adult hypothalamus (17), indicating that transcription in the human brain is initiated at the third promoter site in the IGF-2 gene. A similar transcript has been identified in brain tumors where there is an over-expression of the IGF-2 gene (31).

Protein Products

The protein products of expression of the IGF-2 gene have been identified in the human brain as IGF-2 identical to that first isolated by Rinderknecht and Humbel from serum (7,33), as well as a higher molecular weight form (15). The latter has yet to be sequenced but presumably represents a partially processed form of proIGF-2 which, similar to that isolated from serum, consists of IGF-2 with a carboxyterminus extension peptide. In contract to IGF-1, IGF-2 is found in the cerebrospinal fluid (14). The higher molecular weight form of IGF-2 predominates in human CSF (14) where both forms of IGF-2 are associated with an IGF-2 specific BP (27). Thus, the IGF-2-BP complex may circulate via the CSF to reach the widely distributed IGF-2 receptors.

BIOLOGICAL ACTIVITY

The biological activity of IGF-2 may be mediated via two mechanisms, namely the IGF-1 receptor and the IGF-2/Man-6-P receptor. In purified preparations of human fetal brain, IGF-2 cross-reacts almost equipotently with IGF-1 in the IGF-1 receptor, whereas only IGF-2 cross-reacts in the IGF-2/Man-6-P receptor (28). Based upon studies using blocking receptor antibodies in non-neural cells, it is most likely that IGF-2 induces neuronal and glial cell precursor proliferation by interaction in the IGF-1 receptor. A biological role mediated via the IGF-2/Man-6-P receptor in the brain has yet to be demonstrated. The IGF-2/Man 6-P receptor is widely distributed throughout the brain, and in contract to the IGF-1 receptor, is found in choroid plexus and cerebral vasculature (21,41). Studies in non-neural cells, have implicated the IGF-2/Man-6-P receptor in intracellular protein trafficking and in protein catabolism (20).

Recently, a trophic role of IGF-2 in synaptogenesis has been suggested. Ishii (18) has reported a marked correlation between the expression of the IGF-2 gene in muscle and the rate of neuromuscular synapse formation during synaptogenesis, as well as during muscle reinnervation. Nerve sprouting has been observed following exposure of adult rat gluteus muscle to IGF-2 *in vivo* (9). These studies suggest that IGF-2 may act as a trophic factor from the target cells to induce their innveration. However, further studies to determine the mechanism of this action and the specificity of IGF-2 involvement remain to be performed.

CONCLUSION

The IGFs are synthesized within the CNS to fulfill distinct functions. It has been proposed that the IGF-1 gene is expressed in neurones and glial cells where the protein products, namely truncated IGF-1 and GPE, have an autocrine/paracrine action to regulate growth and modulate neurotransmission, respectively. In contract, IGF-2 is synthesized in choroidal epithelial cells and vascular endothelial cells and in addition to a possible local action on substrate transport, may circulate as the IGF-2-BP complex via the CSF to distal targets within the CNS.

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